Foley, S.
09/482682

(FILE 'CAPLUS' ENTERED AT 08:53:44 ON 07 MAY 2001)
L1 110 SEA ABB=ON PLU=ON (AD OR ADENOVIR? OR ADENO VIR?) AND (TPL OR (TRIPARTITE OR TRI PARTITE)(W)LEADER)
L2 9 SEA ABB=ON PLU=ON L1 AND EXON

L2 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:659615 CAPLUS
DOCUMENT NUMBER: 134:129196
TITLE: Remodeling of the host cell RNA splicing machinery during an **adenovirus** infection
AUTHOR(S): Akusjarvi, Goran
CORPORATE SOURCE: Department of Medical Biochemistry and Microbiology, BMC, Uppsala University, Uppsala, 751 23, Swed.
SOURCE: Recent Res. Dev. Virol. (1999), 1(Pt. 3), 621-630
CODEN: RRDVFH
PUBLISHER: Transworld Research Network
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review, with 49 refs. Topics discussed include: general considerations of **exons**, introns and RNA splicing; temporal control of **adenovirus** L1 unit alternative splicing; function of the IIIa repressor element (3RE); function of the IIIa virus-infection dependent splicing enhancer (3VDE); temporal regulation of **tripartite leader** assembly; and effect of viral DNA replication on alterative RNA splice site choice.
REFERENCE COUNT: 49
REFERENCE(S): (4) Berget, S; Proc Natl Acad Sci U S A 1977, V74, P3171 CAPLUS
(5) Bondesson, M; J Virol 1996, V70, P3844 CAPLUS
(6) Bridge, E; J Virol 1989, V63, P631 CAPLUS
(7) Bruni, R; Proc Natl Acad Sci USA 1996, V93, P10423 CAPLUS
(8) Chen, M; J Biomed Sci 1998, V5, P173 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:493699 CAPLUS
DOCUMENT NUMBER: 133:115933
TITLE: **Adenovirus** vectors with deleted or modified fiber protein and their packaging cell lines for gene therapy
INVENTOR(S): Nemerow, Glen Robert; Von Seggern, Daniel J.; Hallenbeck, Paul L.; Stevenson, Susan C.;

Skripchenko, Yelena
PATENT ASSIGNEE(S): Novartis A.-G., Switz.; Novartis-Erfindungen Verwaltungsgesellschaft m.b.H.; The Scripps Research Institute
SOURCE: PCT Int. Appl., 212 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000042208	A1	20000720	WO 2000-EP265	20000114

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-115920 P 19990114
AB The present invention relates to methods for gene therapy, esp. to **adenovirus**-based gene therapy, and related cell lines and compns. In particular, novel nucleic acid constructs and packaging cell lines are disclosed, for use in facilitating the development of high-capacity and targeted vectors. The invention also discloses a variety of high-capacity **adenovirus** vectors and related compns. and kits including the disclosed cell lines and vectors. Methods of prepg. complementing packaging cell lines for deleted **adenovirus** (**Ad**) vectors by stably transfecting them with plasmids expressing one or more of **adenovirus** E1, E4 or Fiber genes are provided. The helper-independent fiberless **adenovirus** Ad5..beta.gal..DELTA.F has been characterized in terms of genome sequence, replication in complementing cells, compn. and structure of viral particles. These particles are nearly identical to those of first-generation **Ad** vectors with reduced infectivity on epithelial cells, but they retain the ability to infect monocytic cells via an integrin-dependent pathway, which provide a novel approach to developing retargeted **Ad** gene therapy vectors. Targeted **adenovirus** delivery vectors with defective or modified fiber (such as making chimeric fiber of different types **adenoviruses**, inserting heterologous peptide sequence in the H1 loop, adding exogenous fiber , and etc.) and with other modifications including alternative **tripartite**

leader (**TPL**) are also studied. Finally, the invention discloses methods of prepg. and using the disclosed vectors, cell lines and kits.

REFERENCE COUNT: 16

REFERENCE(S):
(1) Chugai Biopharmaceuticals Inc; WO 9737220 A 1997 CAPLUS
(2) Ciba Geigy Ag; WO 9813499 A 1998 CAPLUS
(4) Hardy, S; JOURNAL OF VIROLOGY 1997, V71(3), P1842 CAPLUS
(5) Hoechst Marion Roussel de Gmbh; EP 0892047 A 1999 CAPLUS
(6) Parks, R; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA 1996, V93(24), P13565 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:640455 CAPLUS
DOCUMENT NUMBER: 125:294636
TITLE: Spliced **exons** of **adenovirus** late RNAs colocalize with snRNP in a specific nuclear domain
AUTHOR(S): Bridge, Eileen; Riedel, Kai-Uwe; Johansson, Britt-Marie; Pettersson, Ulf
CORPORATE SOURCE: Dep. Med. Genetics, Uppsala University, Uppsala, S-75123, Swed.
SOURCE: J. Cell Biol. (1996), 135(2), 303-314
CODEN: JCLBA3; ISSN: 0021-9525
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Posttranscriptional steps in the prodn. of mRNA include well characterized polyadenylation and splicing reactions, but it is also necessary to understand how RNA is transported within the nucleus from the site of its transcription to the nuclear pore, where it is translocated to the cytoplasmic compartment. Detg. the localization of RNA within the nucleus is an important aspect of understanding RNA prodn. and may provide clues for investigating the trafficking of RNA within the nucleus and the mechanism for its export to the cytoplasm. We have previously shown that late phase **adenovirus**-infected cells contain large clusters of snRNP and non-snRNP splicing factors; the presence of these structures is correlated with high levels of viral late gene transcriptions. The snRNP clusters correspond to enlarged interchromatin granules present in late phase infected cells. Here we show that polyadenylated RNA and spliced **tripartite leader** **exons** from the major late transcription unit are present in these same late phase snRNP-contg. structures. We find that the majority of the steady state viral RNA present in the nucleus is

spliced at the **tripartite leader exons** . **Tripartite leader exons** are efficiently exported from the nucleus at a time when we detect their accumulation in interchromatin granule clusters. Since the enlarged interchromatin granules contain spliced and polyadenylated RNA, we suggest that viral RNA may accumulate in this late phase structure during an intranuclear step in RNA transport.

L2 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:70815 CAPLUS
DOCUMENT NUMBER: 120:70815
TITLE: Human **adenovirus** encodes two proteins which have opposite effects on accumulation of alternatively spliced mRNAs
AUTHOR(S): Nordqvist, Katarina; Oehman, Karin; Akusjaervi, Goeran
CORPORATE SOURCE: Med. Nobel Inst., Karolinska Inst., Stockholm, S-171 77, Swed.
SOURCE: Mol. Cell. Biol. (1994), 14(1), 437-45
CODEN: MCEBD4; ISSN: 0270-7306
DOCUMENT TYPE: Journal
LANGUAGE: English
AB All mRNAs expressed from the **adenovirus** major late transcription unit have a common, 201-nucleotide-long 5' leader sequence, which consists of three short **exons** (the **tripartite leader**). This leader has two variants, either with or without the i-leader **exon**, which, when present, is spliced between the second and the third **exons** of the **tripartite leader**. Previous studies have shown that **adenovirus** early region 4 (E4) encodes two proteins, E4 open reading frame 3 (E4-ORF3) and E4-ORF6, which are required for efficient expression of mRNAs from the major late transcription unit. These two E4 proteins appear to have redundant activities, and expression of one has been shown to be sufficient for efficient major late mRNA accumulation during a lytic virus infection. In this report, the authors provide evidence that E4-ORF3 and E4-ORF6 both regulate major late mRNa accumulation by stimulating constitutive splicing. Moreover, the authors show that the two proteins have different effects on accumulation of alternatively spliced **tripartite leader exons**. In a DNA transfection assay, E4-ORF3 was shown to facilitate i-leader **exon** inclusion, while E4-ORF6 preferentially favored i-leader **exon** skipping. In addn., E4-ORF3 and E4-ORF6 had the same effects on accumulation of alternatively spliced chimeric .beta.-globin transcripts. This finding suggests that the activities of the two proteins may be of more general relevance and not restricted to splicing of major late **tripartite leader**-contg. pre-mRNAs.

Interestingly, E4-ORF6 expression was also shown to stimulate i-leader **exon** skipping during a lytic virus infection.

L2 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1992:441812 CAPLUS
DOCUMENT NUMBER: 117:41812
TITLE: The 11,600-Mw protein encoded by region E3 of **adenovirus** is expressed early but is greatly amplified at late stages of infection
AUTHOR(S): Tollefson, Ann E.; Scaria, Abraham; Saha, Sankar K.; Wold, William S. M.
CORPORATE SOURCE: Sch. Med., St. Louis Univ., St. Louis, MO, 63110, USA
SOURCE: J. Virol. (1992), 66(6), 3633-42
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors have reported that an 11,600-Mw (11.6K) protein is coded by region E3 of **adenovirus**. In this report two new antipeptide antisera were prepd. that have allowed further characterization of this protein. The 11.6K protein migrates as multiple diffuse bands having apparent Mws of about 14,000, 21,000, and 31,000 on SDS-polyacrylamide gel electrophoresis. Immunoblotting as well as virus mutants with deletions in the 11.6K gene were used to show that the various gel bands represent forms of 11.6K. The 11.6K protein was synthesized in very low amts. during early stages of infection, from the scarce E3 mRNAs d and e which initiate from the E3 promoter. However, 11.6K was synthesized very abundantly at late stages of infection, approx. 400 times the rate at early stages, from new mRNAs termed d' and e'. Reverse transcriptase-polymerase chain reaction and RNA blot expts. indicated that mRNAs d' and e' had the same body (the coding portion) and the same middle **exon** (the y leader) as early E3 mRNAs d and e, but mRNAs d' and e' were spliced at their 5' termini to the major late **tripartite leader** which is found in all mRNAs in the major late transcription unit. MRNAs d' and e' and the 11.6K protein were the only E3 mRNAs and protein that were scarce early and were greatly amplified at late stages of infection. This suggests that specific cis- and trans-acting sequences may function to enhance the splicing of mRNAs d' and e' at late stages of infection and perhaps to suppress the splicing of mRNAs d and e at early stages of infection. The authors propose that the 11.6K gene be considered not only a member of region E3 but also a member of the major late transcription unit.

L2 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:75980 CAPLUS
DOCUMENT NUMBER: 114:75980

TITLE: **Adenovirus** early region 4 stimulates mRNA accumulation via 5' introns
AUTHOR(S): Nordqvist, Katarina; Akusjaervi, Goeran
CORPORATE SOURCE: Med. Nobel Inst., Karolinska Inst., Stockholm, S-104 01, Swed.
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1990), 87(24), 9543-7
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **adenovirus** major late transcription unit accounts for most virus-specific transcription late after infection. All mRNAs expressed from this unit carry a short spliced leader, the so-called **tripartite leader**, attached to their 5' ends. Here, a function is described for an **adenovirus** gene product in the control of major late mRNA abundance. Early region 4 (E4) stimulates mRNA accumulation from **tripartite leader** intron-contg. transcription units .apprx.10-fold in short-term transfection assays. The effect was already detectable in nuclear RNA and was not due to a transcriptional activation through any of the major late promoter elements or through an effect at nuclear-to-cytoplasmic mRNA transport. A surprising positional effect of the intron was noted. To be E4-responsive, the intron had to be placed close to the pre-mRNA 5' end. The same intron located far downstream in the 3'-untranslated region of the mRNA was not E4 responsive. The E4 enhancement was not dependent on specific virus **exon** or intron sequences. These results suggest that E4 modulates a general pathway in mammalian mRNA formation.

L2 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1986:201576 CAPLUS
DOCUMENT NUMBER: 104:201576
TITLE: Human factor VIII, compositions containing it, methods and materials for use in its production
INVENTOR(S): Capon, Daniel Jeffrey; Vehar, Gordon Allen; Lawn, Richard Mark; Wood, William Irwin
PATENT ASSIGNEE(S): Genentech, Inc., USA
SOURCE: Eur. Pat. Appl., 110 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 160457	A1	19851106	EP 1985-302734	19850418
EP 160457	B1	19910116		

Searcher : Shears 308-4994

R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
FI 8501497	A	19851021	FI 1985-1497	19850415
FI 86885	B	19920715		
FI 86885	C	19921026		
IL 74909	A1	19920115	IL 1985-74909	19850415
IL 93200	A1	19920115	IL 1985-93200	19850415
IL 93201	A1	19920115	IL 1985-93201	19850415
ES 542296	A1	19860601	ES 1985-542296	19850416
AU 8541345	A1	19851024	AU 1985-41345	19850417
AU 601358	B2	19900913		
HU 37654	A2	19860123	HU 1985-1428	19850417
HU 202275	B	19910228		
ZA 8502864	A	19861230	ZA 1985-2864	19850417
NO 8501563	A	19851021	NO 1985-1563	19850418
NO 174934	B	19940425		
NO 174934	C	19940803		
EP 385558	A2	19900905	EP 1990-200797	19850418
EP 385558	A3	19901227		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
AT 60087	E	19910215	AT 1985-302734	19850418
DK 8501768	A	19851021	DK 1985-1768	19850419
DK 164876	B	19920831		
DK 164876	C	19930118		
JP 60243023	A2	19851203	JP 1985-85295	19850419
JP 2799338	B2	19980917		
US 4965199	A	19901023	US 1987-83758	19870807
AU 9052958	A1	19900830	AU 1990-52958	19900405
US 5618788	A	19970408	US 1990-570096	19900820
US 5633150	A	19970527	US 1990-595481	19901009
US 5618789	A	19970408	US 1992-829867	19920203
JP 06040942	A2	19940215	JP 1993-93222	19930420
JP 2777043	B2	19980716		
US 5654147	A	19970805	US 1995-447486	19950523
US 5668108	A	19970916	US 1995-447654	19950523
US 5683905	A	19971104	US 1995-448171	19950523

PRIORITY APPLN. INFO.:	US 1984-602312	19840420
	IL 1985-74909	19850415
	EP 1985-302734	19850418
	US 1987-83758	19870807
	US 1990-570096	19900820
	US 1990-595481	19901009
	US 1992-829867	19920203

AB The full cDNA sequence of human blood-coagulation factor VIII is presented and an expression vector that is capable of directing the synthesis of human factor VIII in baby hamster kidney (BHK) cells is prepd. Thus, plasmid pAML3P.8c1 comprised the **adenovirus tripartite leader** spliced in the 3rd **exon** to the 5' untranslated region of the factor VIII sequence followed

by the full-length factor VIII structural gene, including its signal sequence. The 3' untranslated region of the factor VIII gene was spliced to the 3' untranslated region of the hepatitis B surface antigen gene. This was followed by the dihydrofolate reductase gene which has an SV40 virus early promoter and a hepatitis virus 3' untranslated region conferring a functional polyadenylation signal. The factor VIII expression plasmid, pAML3P.8c1, was contransfected into BHK cells with the neomycin resistance vector pSVEneoBal6. These cells were first selected with G418 followed by selection with methotrexate. RIAs were performed on supernatants and lysed cells of the BHK factor VIII-producing cell lines to det. the amt. of factor VIII produced by the cells.

L2 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1985:126448 CAPLUS
DOCUMENT NUMBER: 102:126448
TITLE: Identification of the components necessary for **adenovirus** translational control and their utilization in cDNA expression vectors
AUTHOR(S): Kaufman, Randal J.
CORPORATE SOURCE: Genet. Inst., Boston, MA, 02115, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1985), 82(3), 689-93
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A transient expression system was used to study the role of the **adenovirus** late and SV40 virus early mRNA leader sequences and **adenovirus** virus-assocd. (VA) RNAs in mRNA translation. Hybrid transcription units contg. the **adenovirus** late and SV40 early promoters fused to various coding regions were introduced into monkey COS cells on plasmids contg. a SV40 origin of replication. The translational efficiencies of the mRNAs produced from these plasmids were detd. after alterations in the viral leader sequences or in the presence of VA RNAs provided by **adenovirus** infection of the transfected cells or by cotransfection with plasmids contg. the VA genes. Efficient translation of mRNA with either **adenovirus** or SV40 leader sequences is dependent upon the presence of VA RNA. Translational stimulation by VA RNA of mRNAs contg. the **adenovirus tripartite leader** sequences is dramatically reduced if leader **exons** 2 and 3 are removed or if their orientation is altered. Sequence anal. has indicated a homol. between the nontranslated 5' end of SV40 early mRNA and sequences at the border of the 2nd and 3rd **tripartite leader exons**, which may be responsible for the increased translation of these mRNAs in the presence of VA RNA.

L2 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1983:13313 CAPLUS
DOCUMENT NUMBER: 98:13313
TITLE: Sequence dependent interaction of hnRNP proteins with late **adenoviral** transcripts
AUTHOR(S): Van Eekelen, Chris; Ohlsson, Rolf; Philipson, Lennart; Mariman, Edwin; Van Beek, Rita; Van Venrooij, Walther
CORPORATE SOURCE: Dept. Biochem., Univ. Nijmegen, Nijmegen, 6525 EZ, Neth.
SOURCE: Nucleic Acids Res. (1982), 10(22), 7115-31
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Irradn. with UV light was used to induce covalent linkage between heterogeneous nuclear RNA (hnRNA) and its assocd. proteins in intact HeLa cells late after infection with **adenovirus** type 2. Covalently linked hnRNA-protein complexes (hnRNP) contg. **adenovirus** poly(A)+ RNA were isolated and their protein moieties were characterized. Host 42,000-dalton hnRNP proteins were the major proteins crosslinked to viral hnRNA. To investigate their possible involvement in RNA processing, the localization of these crosslinked polypeptides on **adenoviral** late transcripts was detd. Sequences of RNA around the attachment sites of the protein were isolated. After in vitro labeling they were hybridized to Southern blots of **adenovirus** DNA fragments. The hybridization patterns revealed that the 42,000-dalton polypeptides can be linked to **adenoviral** transcripts over the entire length of the RNA, corresponding to 16.2-91.5 map units of the viral genome. Fine mapping within the HindIII B region (16.8-31.5 map units) established, however, that the localization of the crosslinked polypeptides was not random in all parts of the transcript. Sequences around the 3rd leader and the 3' part of the i-leader were overrepresented, whereas the regions encoding VA I and VA II RNA and the late region 1 mRNA bodies were underrepresented in the crosslinked RNA. Using genomic DNA fragments and a cDNA clone contg. the **tripartite leader**, it appeared that leader and intervening sequences were represented about equally in crosslinked RNA fragments. Although these results do not support the notion that introns or **exons** are specifically interacting with 1 RNP protein, they demonstrate that the 42,000-dalton hnRNP proteins are nonrandomly positioned on the RNA sequence.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 08:58:54 ON 07 MAY 2001)
L3 20 S L2

L4 7 DUP REM L3 (13 DUPLICATES REMOVED)

L4 ANSWER 1 OF 7 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-476068 [41] WPIDS
DOC. NO. CPI: C2000-142803
TITLE: New nucleic acid comprising an **adenovirus tripartite leader** nucleotide for producing high-capacity and targeted vectors for **adenovirus**-based gene therapy.
DERWENT CLASS: B04 D16
INVENTOR(S): HALLENBECK, P L; NEMEROW, G R; SKRIPCHENKO, Y; STEVENSON, S C; VON SEGGERN, D J
PATENT ASSIGNEE(S): (NEME-I) NEMEROW G R; (NOVS) NOVARTIS AG; (NOVS) NOVARTIS-ERFINDUNGEN VERW GES MBH; (SCRI) SCRIPPS RES INST
COUNTRY COUNT: 89
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000042208	A1	20000720	(200041)*	EN	212
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2000024372	A	20000801	(200054)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000042208	A1	WO 2000-EP265	20000114
AU 2000024372	A	AU 2000-24372	20000114

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000024372	A Based on	WO 200042208

PRIORITY APPLN. INFO: US 1999-115920 19990114
AN 2000-476068 [41] WPIDS
AB WO 200042208 A UPAB: 20000831
NOVELTY - An isolated nucleic acid molecule (I) comprising an **adenovirus** (AV) **tripartite leader** (**TPL**) nucleotide with a sequence comprising two different

TPL exons or three same or different **TPL exons**, is new.

DETAILED DESCRIPTION - A new isolated nucleic acid molecule (I) comprises an AV **TPL** nucleotide with a sequence comprising **TPL exons** that are complete **TPL exon** 1, partial **TPL exon** 1, complete **TPL exon** 2 or complete **TPL exon** 3 and which may not comprise partial **TPL exon** 1, complete **TPL exon** 2 and complete **TPL exon** 3.

INDEPENDENT CLAIMS are also included for the following:

(1) an AV vector complementing plasmid comprising (I);

(2) an AV vector packaging cell line (PCL) comprising (I), a promoter and a nucleic acid that encodes an AV structural protein, where the **TPL** sequence consists of a complete **TPL exon** linked to a complete second **TPL exon** linked to a complete third **TPL exon**;

(3) a recombinant AV particle (RAP) comprising a recombinant AV vector genome (RAVG) that does not encode or express sufficient AV fiber protein (FP) to support packaging of a fiber-containing AV without complementation of the fiber gene in a PC comprising the **TPL** nucleotide sequence of the new nucleic acid, and optionally an exogenous protein;

(4) a helper-independent fiberless RAVG comprising genes which: encode all AV structural gene products but do not express sufficient AV FP to package a fiber-containing AV particle in a PC containing a **TPL** nucleotide sequence of (I), without complementation of the fiber gene or the genome lacks the fiber gene; or encode an exogenous protein;

(5) an isolated nucleic acid that comprises (4);

(6) producing an AV vector particle containing (4) comprising providing a PCL (containing (I)) which complements replication and packaging of the genome and (4) which is deficient in expressing functional FP to support assembly of fiber-containing particles, and harvesting the particles produced by the CL;

(7) delivering an exogenous gene to a target cell comprising infecting the cell with a RAP;

(8) pseudotyping recombinant viral vectors comprising complementing a missing fiber gene of a helper-independent or helper-dependent fiberless RAVG by expressing in PC's a fiber gene from a different **adenoviral** serotype than the RAV;

(9) targeting an AV vector to a cell comprising introducing a helper-independent or -dependent fiberless RAVG into a PCL for producing a fiber gene-deleted AV vector, where the gene for a missing FP is complemented with a gene for a desired modification;

(10) producing a modified AV comprising providing in vitro an exogenous FP to a fiberless virus preparation;

(11) delivering a heterologous gene to Epstein-Barr virus

(EBV)-infected B cells comprising infecting the cells with a pseudotyped Ad5 beta gal. Delta F particle or other fiber-deleted AV particle having a chimeric fiber including the receptor-binding knob domain of the AV type 3 fiber;

(12) producing a modified AV comprising producing a fiberless AV helper-dependent fiberless RAVG in a PCL and a helper virus vector, where the CL complements a deficient FP gene;

(13) a composition for preparing a therapeutic vector comprising a plasmid containing an AV genome lacking a nucleotide sequence encoding a FP or a genome incapable of expressing fiber which results in packaging;

(14) delivering a heterologous gene to an animal comprising contacting a target cell in vivo or ex vivo with a RAP that infects the cell;

(15) producing a gutless **adenoviral** vector particle comprising:

(a) delivering a helper AV vector genome that lacks any gene encoding AV FP or lacks the ability to encode AV FP to produce an **adenoviral** vector comprising FP in the absence of complementation by the PC (comprising (I) linked to a promoter and to an **adenoviral** FP or chimeric protein including an AV FP tail domain) to an AV vector PC;

(b) delivering a gutless AV vector genome to the PC; and recovering the gutless **adenoviral** vector particle produced by the cell;

(16) a helper AV particle comprising an AV vector genome with a mutation in its packaging sequence that renders it incapable of being packaged, that does not encode or express AV protein to support packaging of a fiber-containing AV particle without complementation of the fiber gene;

(17) a helper AV particle comprising an AV vector genome with recombinase sites flanking its packaging sequence, where the vector genome does not encode or express AV fiber protein to support packaging of a fiber-containing AV particle without complementation of the fiber gene;

(18) an AV particle comprising a gutless **adenoviral** vector genome and a fiberless capsid;

(19) an AV particle comprising a gutless **adenoviral** vector genome and a capsid with a modified FP; and

(20) a PC for the production of a fiberless or fiber-modified gutless AV particle comprising an AV vector complementing plasmid (containing (I) linked to a promoter and a nucleotide sequence encoding an **adenoviral** FP or chimeric **adenoviral** FP) and a nucleotide sequence encoding a recombinase.

ACTIVITY - None given.

MECHANISM OF ACTION - Gene therapy. No biological data is given.

USE - (I) is used to produce an AV vector particle, deliver an

exogenous gene to a target cell, pseudotype recombinant viral vectors, target an AV vector to a cell, produce a modified AV, deliver a heterologous gene to an animal and produce a gutless **adenoviral** vector particle (claimed).
Dwg.0/30

L4 ANSWER 2 OF 7 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 97051955 MEDLINE
DOCUMENT NUMBER: 97051955 PubMed ID: 8896590
TITLE: Spliced **exons** of **adenovirus** late RNAs colocalize with snRNP in a specific nuclear domain.
AUTHOR: Bridge E; Riedel K U; Johansson B M; Pettersson U
CORPORATE SOURCE: Department of Medical Genetics, Uppsala University, Biomedical Center, Sweden..
eileen.bridge@medgen.uu.se
SOURCE: JOURNAL OF CELL BIOLOGY, (1996 Oct) 135 (2) 303-14.
Journal code: HMV; 0375356. ISSN: 0021-9525.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961205

AB Posttranscriptional steps in the production of mRNA include well characterized polyadenylation and splicing reactions, but it is also necessary to understand how RNA is transported within the nucleus from the site of its transcription to the nuclear pore, where it is translocated to the cytoplasmic compartment. Determining the localization of RNA within the nucleus is an important aspect of understanding RNA production and may provide clues for investigating the trafficking of RNA within the nucleus and the mechanism for its export to the cytoplasm. We have previously shown that late phase **adenovirus**-infected cells contain large clusters of snRNP and non-snRNP splicing factors; the presence of these structures is correlated with high levels of viral late gene transcription. The snRNP clusters correspond to enlarged interchromatin granules present in late phase infected cells. Here we show that polyadenylated RNA and spliced **tripartite leader exons** from the viral major late transcription unit are present in these same late phase snRNP-containing structures. We find that the majority of the steady state viral RNA present in the nucleus is spliced at the **tripartite leader exons**. **Tripartite leader exons** are efficiently exported from the nucleus at a time when we detect their accumulation in interchromatin granule clusters. Since the

enlarged interchromatin granules contain spliced and polyadenylated RNA, we suggest that viral RNA may accumulate in this late phase structure during an intranuclear step in RNA transport.

L4 ANSWER 3 OF 7 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 94088542 MEDLINE
DOCUMENT NUMBER: 94088542 PubMed ID: 8264611
TITLE: Human **adenovirus** encodes two proteins which have opposite effects on accumulation of alternatively spliced mRNAs.
AUTHOR: Nordqvist K; Ohman K; Akusjarvi G
CORPORATE SOURCE: Department of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institutet, Stockholm, Sweden.
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1994 Jan) 14 (1) 437-45.
Journal code: NGY; 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940209
Last Updated on STN: 19970203
Entered Medline: 19940121

AB All mRNAs expressed from the **adenovirus** major late transcription unit have a common, 201-nucleotide-long 5' leader sequence, which consists of three short **exons** (the **tripartite leader**). This leader has two variants, either with or without the i-leader **exon**, which, when present, is spliced between the second and the third **exons** of the **tripartite leader**. Previous studies have shown that **adenovirus** early region 4 (E4) encodes two proteins, E4 open reading frame 3 (E4-ORF3) and E4-ORF6, which are required for efficient expression of mRNAs from the major late transcription unit. These two E4 proteins appear to have redundant activities, and expression of one has been shown to be sufficient for efficient major late mRNA accumulation during a lytic virus infection. In this report, we provide evidence that E4-ORF3 and E4-ORF6 both regulate major late mRNA accumulation by stimulating constitutive splicing. Moreover, we show that the two proteins have different effects on accumulation of alternatively spliced **tripartite leader exons**. In a DNA transfection assay, E4-ORF3 was shown to facilitate i-leader **exon** inclusion, while E4-ORF6 preferentially favored i-leader **exon** skipping. In addition, E4-ORF3 and E4-ORF6 had the same effects on accumulation of alternatively spliced chimeric beta-globin transcripts. This finding suggests that the

activities of the two proteins may be of more general relevance and not restricted to splicing of major late **tripartite leader**-containing pre-mRNAs. Interestingly, E4-ORF6 expression was also shown to stimulate i-leader **exon** skipping during a lytic virus infection.

L4 ANSWER 4 OF 7 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 92260635 MEDLINE
DOCUMENT NUMBER: 92260635 PubMed ID: 1316473
TITLE: The 11,600-MW protein encoded by region E3 of **adenovirus** is expressed early but is greatly amplified at late stages of infection.
AUTHOR: Tollefson A E; Scaria A; Saha S K; Wold W S
CORPORATE SOURCE: Institute for Molecular Virology, St. Louis University School of Medicine, Missouri 63110.
CONTRACT NUMBER: CA24710 (NCI)
SOURCE: JOURNAL OF VIROLOGY, (1992 Jun) 66 (6) 3633-42. Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920626
Last Updated on STN: 19920626
Entered Medline: 19920616

AB We have reported that an 11,600-MW (11.6K) protein is coded by region E3 of **adenovirus**. We have now prepared two new antipeptide antisera that have allowed us to characterize this protein further. The 11.6K protein migrates as multiple diffuse bands having apparent Mws of about 14,000, 21,000, and 31,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunoblotting as well as virus mutants with deletions in the 11.6K gene were used to show that the various gel bands represent forms of 11.6K. The 11.6K protein was synthesized in very low amounts during early stages of infection, from the scarce E3 mRNAs d and e which initiate from the E3 promoter. However, 11.6K was synthesized very abundantly at late stages of infection, approximately 400 times the rate at early stages, from new mRNAs termed d' and e'. Reverse transcriptase-polymerase chain reaction and RNA blot experiments indicated that mRNAs d' and e' had the same body (the coding portion) and the same middle **exon** (the y leader) as early E3 mRNAs d and e, but mRNAs d' and e' were spliced at their 5' termini to the major late **tripartite leader** which is found in all mRNAs in the major late transcription unit. mRNAs d' and e' and the 11.6K protein were the only E3 mRNAs and protein that were scarce early and were greatly amplified at late stages of infection. This suggests that specific cis- or

trans-acting sequences may function to enhance the splicing of mRNAs d' and e' at late stages of infection and perhaps to suppress the splicing of mRNAs d and e at early stages of infection. We propose that the 11.6K gene be considered not only a member of region E3 but also a member of the major late transcription unit.

L4 ANSWER 5 OF 7 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 91088554 MEDLINE
DOCUMENT NUMBER: 91088554 PubMed ID: 2263609
TITLE: **Adenovirus** early region 4 stimulates mRNA accumulation via 5' introns.
AUTHOR: Nordqvist K; Akusjarvi G
CORPORATE SOURCE: Department of Microbial Genetics, Karolinska Institutet, Stockholm, Sweden.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1990 Dec) 87 (24) 9543-7.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199102
ENTRY DATE: Entered STN: 19910322
Last Updated on STN: 19910322
Entered Medline: 19910207

AB The **adenovirus** major late transcription unit accounts for most virus-specific transcription late after infection. All mRNAs expressed from this unit carry a short spliced leader, the so-called **tripartite leader**, attached to their 5' ends. Here we describe a function for an **adenovirus** gene product in the control of major late mRNA abundance. We show that early region 4 (E4) stimulates mRNA accumulation from **tripartite leader** intron-containing transcription units approximately 10-fold in short-term transfection assays. The effect was already detectable in nuclear RNA and was not due to a transcriptional activation through any of the major late promoter elements or through an effect at nuclear to cytoplasmic mRNA transport. A surprising positional effect of the intron was noted. To be E4 responsive, the intron had to be placed close to the pre-mRNA 5' end. The same intron located far downstream in the 3' untranslated region of the mRNA was not E4 responsive. The E4 enhancement was not dependent on specific virus **exon** or intron sequences. These results suggest that E4 modulates a general pathway in mammalian mRNA formation.

L4 ANSWER 6 OF 7 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 85140162 MEDLINE

DOCUMENT NUMBER: 85140162 PubMed ID: 2983309
TITLE: Identification of the components necessary for **adenovirus** translational control and their utilization in cDNA expression vectors.
AUTHOR: Kaufman R J
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1985 Feb) 82 (3) 689-93.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198503
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19980206
Entered Medline: 19850327

AB A transient expression system was used to study the role of the **adenovirus** late and simian virus 40 (SV40) early mRNA leader sequences and **adenovirus** virus-associated (VA) RNAs in mRNA translation. Hybrid transcription units containing the **adenovirus** late and SV40 early promoters fused to various coding regions were introduced into monkey COS cells on plasmids containing a SV40 origin of replication. The translational efficiencies of the mRNAs produced from these plasmids were determined after alterations in the viral leader sequences or in the presence of VA RNAs provided by **adenovirus** infection of the transfected cells or by cotransfection with plasmids containing the VA genes. Efficient translation of mRNA with either **adenovirus** or SV40 leader sequences is dependent upon the presence of VA RNA. Translational stimulation by VA RNA of mRNAs containing the **adenovirus tripartite leader** sequences is dramatically reduced if leader **exons** 2 and 3 are removed or if their orientation is altered. Sequence analysis has indicated a homology between the nontranslated 5' end of SV40 early mRNA and sequences at the border of the 2nd and 3rd **tripartite leader exons**, which may be responsible for the increased translation of these mRNAs in the presence of VA RNA.

L4 ANSWER 7 OF 7 MEDLINE
ACCESSION NUMBER: 83116943 MEDLINE
DOCUMENT NUMBER: 83116943 PubMed ID: 6296766
TITLE: Sequence dependent interaction of hnRNP proteins with late **adenoviral** transcripts.
AUTHOR: van Eekelen C; Ohlsson R; Philipson L; Mariman E; van Beek R; van Venrooij W
SOURCE: NUCLEIC ACIDS RESEARCH, (1982 Nov 25) 10 (22)

7115-31.
Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198303
ENTRY DATE: Entered STN: 19900318
Last Updated on STN: 19970203
Entered Medline: 19830324

AB Irradiation with ultraviolet light was used to induce covalent linkage between hnRNA and its associated proteins in intact HeLa cells, late after infection with **adenovirus** type 2. Covalently linked hnRNA-protein complexes, containing polyadenylated **adenoviral** RNA, were isolated and their protein moiety characterized. Host 42,000 Mr hnRNP proteins proved to be the major proteins crosslinked to viral hnRNA. To investigate their possible involvement in RNA processing, the localization of these cross-linked polypeptides on **adenoviral** late transcripts was determined. Sequences of RNA around the attachment sites of the protein were isolated. After in vitro labeling they were hybridized to Southern blots of adeno DNA fragments. The hybridization patterns revealed that the 42,000 Mr polypeptides can be linked to **adenoviral** transcripts over the entire length of the RNA, corresponding to 16.2-91.5 m.u. of the viral genome. Fine mapping within the Hind III B region (16.8-31.5 m.u.) established, however, that the localization of the cross-linked polypeptides was not random in all parts of the transcript. Sequences around the third leader and the 3' part of the i-leader were overrepresented, whereas the regions encoding VA I and VA II RNA and the late region 1 mRNA bodies were underrepresented in the cross-linked RNA. Using genomic DNA fragments and a cDNA clone containing the **tripartite leader** it appeared that leader and intervening sequences were represented about equally in cross-linked RNA fragments. Although these results do not support the notion that introns or **exons** are specifically interacting with one RNP protein, they demonstrate that the 42,000 hnRNP proteins are non randomly positioned on the RNA sequence.

FILE 'CAPLUS' ENTERED AT 09:00:09 ON 07 MAY 2001
L5 30 SEA ABB=ON PLU=ON (AD OR ADENOVIR? OR ADENO VIR?)(W)(TP L OR (TRIPARTITE OR TRI PARTITE)(W)LEADER)
L6 27 SEA ABB=ON PLU=ON L5 NOT L2

L6 ANSWER 1 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:414683 CAPLUS
DOCUMENT NUMBER: 134:69966
TITLE: Gene immunization of mice with plasmid DNA

expressing rabies virus glycoprotein

AUTHOR(S): Fodor, I.; Kucsera, L.; Fodor, Nadja; Palfi, V.; Grabko, V. I.

CORPORATE SOURCE: Agricultural Biotechnology Center, Godollo, H-2101, Hung.

SOURCE: Acta Vet. Hung. (2000), 48(2), 229-236
CODEN: AVHUEA; ISSN: 0236-6290

BLISHER: Akademiai Kiado

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Gene immunization can be an effective vaccine strategy eliciting both humoral and cell-mediated immune responses. We constructed plasmid vectors expressing the full-length Vnukovo-32 rabies virus glycoprotein G under the control of CMV IE promoter and enhancer, **adenovirus tripartite leader** sequences and poly A signal of SV40. The gene vaccines were evaluated for the ability to elicit neutralizing antibodies and to protect BALB/c mice against lethal rabies virus challenge. First, mice were injected i.m. into the left hind leg and by the intradermoplantar (i.d.p.) route with equal amts. of plasmid DNA (0.25-0.1 mg). Two weeks later, immunization was boosted with an addnl. dose of the DNA. The immunized mice were challenged by intracerebral (i.c.) inoculation of CVS-27 (10-50 LD50) rabies virus. All mice produced anti-rabies virus neutralizing antibodies with a titer of .gtoreq. 1:45 after immunization with 0.1-0.4 mg of DNA. In challenge expts., 83 to 91.6% protection was obsd. These results confirm that a DNA vaccine could be a simple and effective soln. for preventing the spread of rabies.

REFERENCE COUNT: 37

REFERENCE(S):

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(3) Bunschoten, H; J Gen Virol 1989, V70, P291 CAPLUS

(5) Conzelmann, K; Virology 1990, V175, P485 CAPLUS

(6) Coulon, P; J Gen Virol 1982, V61, P97 CAPLUS

(8) Cox, G; J Virol 1993, V67, P5664 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 27 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:243480 CAPLUS

DOCUMENT NUMBER: 133:236560

TITLE: Induction of protective immunity in chickens immunised with plasmid DNA encoding infectious bursal disease virus antigens

AUTHOR(S): Fodor, I.; Horvath, E.; Fodor, Nadja; Nagy, Edith; Rencendorsh, Altancsimeg; Vakharia, V. N.; Dube, S. K.

CORPORATE SOURCE: Agricultural Biotechnology Centre, Godollo, H-2100, Hung.
SOURCE: Acta Vet. Hung. (1999), 47(4), 481-492
CODEN: AVHUEA; ISSN: 0236-6290
PUBLISHER: Akademiai Kiado
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Direct DNA inoculations were used to det. the efficacy of gene immunization of chickens to elicit protective immune responses against infectious bursal disease virus (IBDV). The vp2 gene of IBDV strains GP40 and D78, and the vp2-vp4-vp3 encoding segment of strain D78 were cloned in an expression vector which consisted of human cytomegalovirus (HCMV) immediate early enhancer and promoter, **adenovirus tripartite leader** sequences and SV40 polyadenylation signal. For purifn. of vaccine-quality plasmid DNA from E. coli, an effective method was developed. Chickens were vaccinated by inoculation of DNA by two routes (i.m. and i.p.). Two weeks later, chickens were boosted with DNA, and at 2 wk post-boost, they were challenged with virulent IBDV strain. Low to undetectable levels of IBDV-specific antibodies and no protection were obsd. with DNA encoding VP2. However, plasmids encoding VP2-VP4-VP3 induced IBDV-specific antibodies and protection in the chickens. DNA immunization opens a new approach to the development of gene vaccines for chickens against infectious diseases.

REFERENCE COUNT: 19
REFERENCE(S):
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:570336 CAPLUS
DOCUMENT NUMBER: 130:11208
TITLE: Ribosome shunting: selective translation initiation conferred by the **adenovirus tripartite leader** 5' non-coding region in adenovirus-infected and heat-shocked cells
AUTHOR(S): Yueh, Yueh Andrew
CORPORATE SOURCE: New York Univ., New York, NY, USA
SOURCE: (1998) 177 pp. Avail.: UMI, Order No. DA9831785 From: Diss. Abstr. Int., B 1998, 59(4), 1513
DOCUMENT TYPE: Dissertation

LANGUAGE: English
AB Unavailable

L6 ANSWER 4 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:114747 CAPLUS
DOCUMENT NUMBER: 128:191672
TITLE: Development of high output expression vectors for antibody production in mammalian cells
AUTHOR(S): Gervais, Christian; Paquette, Denis; Burns-Tardif, Ann; Martin, Luis; Massie, Bernard
CORPORATE SOURCE: Biomira inc., Montreal, H4P 2R2, Can.
SOURCE: Anim. Cell Technol.: Basic Appl. Aspects, Proc. Annu. Meet. Jpn. Assoc. Anim. Cell Technol., 9th (1998), Meeting Date 1996, 349-354. Editor(s): Nagai, Kazuo; Wachi, Masaaki. Kluwer: Dordrecht, Neth.
CODEN: 65RGAA
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The design of efficient expression vectors is a major component in the development of a high level prodn. system for recombinant antibodies. The BKV enhancer/Adenovirus major-late promoter (BKV/AdMLP) and human cytomegalovirus immediate-early enhancer/promoter (CMV) were evaluated. The **Adenovirus tripartite leader** contg. a chimeric intron (AdTpl-SS), Ig leader intron and J/C chimeric intron were added and/or shifted. Cell lines COS-1, CV-1, 293S, CHO and NSO were used as hosts for transient assays. The secreted products were quantified by ELISA. In all cell lines, the CMV enhancer/promoter yielded higher secretion levels than the BKV/AdMLP. AdTpl-SS and J/C intron each had a pos. but not cumulative effect whereas splicing the Ig leader region only complicated the cloning and was without significant benefit. Positioning the J/C intron between the variable and the const. domain is necessary to obtain benefits except in CHO where the same intron inserted downstream of a cDNA yielded comparative results. Overall, adding splice site(s) to cDNA increased secretion 2 to 6 times. In transient expression, levels of 8 to 17 .mu.g of Kappa chain per mL were measured resp. in COS-1 and in 293S cells using our best construct. Results obtained from transient assays led to the assembly of two cassettes in tandem to express both chains of B43.13 MAb. Stable expression levels in static batch cultures attain 80 .mu.g/mL in 293S and over 350 .mu.g/mL in NSO using serum-free medium.

L6 ANSWER 5 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:596551 CAPLUS
DOCUMENT NUMBER: 127:276845
TITLE: Comparison of various expression plasmids for

the induction of immune response by DNA immunization

AUTHOR(S): Lee, Ann Hwee; Suh, You Suk; Sung, Jun Ho; Yang, Se Hwan; Sung, Young Chul

CORPORATE SOURCE: Department of Life Science, School of Environmental Engineering, Pohang University of Science and Technology, Pohang, 790-784, S. Korea

SOURCE: Mol. Cells (1997), 7(4), 495-501
CODEN: MOCEEK; ISSN: 1016-8478

PUBLISHER: Korean Society of Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB I.m. injection of plasmid DNA is an efficient method to introduce a foreign gene into a live animal. The authors investigated several factors affecting the gene transfer efficiency and the following immune response by i.m. injection of plasmid DNA. When the strength of several highly efficient viral promoters was compared in muscle by using the chloramphenicol acetyltransferase (CAT) gene as an indicator, cytomegalovirus (CMV) immediate early promoter was stronger than any other viral promoters including Rous sarcoma virus (RSV), murine leukemia virus (SL3-3), and simian virus 40 (SV40) early promoters. Inclusion of **adenovirus tripartite leader** (TPL) sequences and a synthetic intron in the 5' untranslated region of mRNA moderately stimulated the CAT expression. The expression of encephalomyocarditis virus (EMCV) VP1 gene was greatly enhanced by the TPL sequences and an intron. The level of humoral immune response by i.m. injection of various VP1 expression plasmids was compared. The seroconversion rate was highly dependent on the strength of the expression vector. However, the ratio of IgG1 and IgG2a immune response was not variable depending on the strength of the expression vector. Also, the efficiency of the sindbis virus-based DNA vector was examd. for the gene expression and immune response. Although a high level of CAT expression was obtained in muscle by using this system, VP1 was not produced as much as the conventional expression vectors. Furthermore, little humoral immune response was elicited by i.m. injection of VP1-expressing sindbis vector, suggesting that this system was not superior to the conventional vector for DNA immunization.

L6 ANSWER 6 OF 27 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:633284 CAPLUS

DOCUMENT NUMBER: 126:1999

TITLE: Nucleotide sequence of ovine **adenovirus tripartite leader** sequence and homologues of the IVa2, DNA polymerase and terminal proteins

AUTHOR(S): Vrati, Sudhanshu; Brookes, D. E.; Boyle, D. B.; Both, G. W.
CORPORATE SOURCE: CSIRO Division of Biomolecular Engineering, P.O. Box 184, North Ryde, N.S.W., 2113, Australia
SOURCE: Gene (1996), 177(1/2), 35-41
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Ovine adenovirus OAV287 was previously isolated from sheep in Western Australia. Here we describe a portion of its genome between map units 10.3 and 31.7 which includes major ORFs for homologues of the IVa2 polypeptide and the DNA replication proteins, Terminal protein and DNA polymerase, as well as the N-terminal portion of the 52/55-kDa polypeptide. In addn., as a prelude to possible adaptation of this virus as a vector we have mapped the elements which make up the tripartite leader sequence of late mRNAs, thereby defining the probable location of the OAV major late promoter. In other human and animal adenovirus genomes, one or two VA RNA genes are encoded between the ORFs for Terminal protein and 52/55-kDa polypeptides. In OAV, these ORFs overlap, suggesting that if VA RNA genes are present, they may lie elsewhere in the OAV genome.

L6 ANSWER 7 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:599233 CAPLUS
DOCUMENT NUMBER: 125:240240
TITLE: Transfer vector for preparation of adenovirus expression vectors for high-level recombinant protein production
INVENTOR(S): Massie, Bernard; Langelier, Yves; Lamarche, Natalie
PATENT ASSIGNEE(S): National Research Council of Canada, Can.
SOURCE: U.S., 23 pp. Cont.-in-part of U.S. Ser. No. 58,800,abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5518913	A	19960521	US 1994-232998	19940422
CA 2053187	AA	19930411	CA 1991-2053187	19911010

PRIORITY APPLN. INFO.: CA 1991-2053187 19911010
US 1991-774223 19911010
US 1993-58800 19930510

AB The present invention relates to methods and compns. involving an

improved recombinant transfer vector for introducing a DNA sequence, encoding a recombinant protein, into an adenovirus genome in generating recombinant adenovirus. Recombinant protein prodn., in cells infected with the recombinant adenovirus, can approach levels as high as 15-20% of total cellular proteins. The improved transfer vector includes an expression cassette comprising sequentially a transcription promoter, a high efficiency leader, at least one splicing signal, an enhancer-like sequence, a cloning site and a plurality of polyadenylation sites. Transfer vector pAdBM5, for prepn. of recombinant adenovirus 5 expression vectors by homologous recombination, was constructed. The expression cassette of pAdBM5 comprises the BK virus enhancer-like sequence, the adenovirus MLP promoter, the **adenovirus tripartite leader**, and a second enhancer-like region consisting of adenovirus binding sites R1, R2, and R3. The position of the first enhancer-like sequence was crit. in enhancing expression from the MLP. Recombinant adenovirus prepd. with this transfer vector were used to infect 293 cells and recombinant cells were cultured to produce herpes simplex virus ribonucleotide reductase subunits.

L6 ANSWER 8 OF 27 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:	1995:572952 CAPLUS
DOCUMENT NUMBER:	123:26596
TITLE:	Highly efficient eukaryotic gene expression vectors for peptide secretion
AUTHOR(S):	Chu, Te-Hua Tearina; Martinez, Idali; Olson, Paul; Dornburg, Ralph
CORPORATE SOURCE:	Rutgers Univ. Medicine Dentistry, Piscataway, NJ, USA
SOURCE:	Pept. Res. (1995), 8(2), 101-7 CODEN: PEREEO; ISSN: 1040-5704
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Recently, we constructed a series of highly efficient universal eukaryotic gene expression vectors (Sheay et al., BioTechniques 15:856-862, 1993). Such vectors contain a viral promoter and enhancer followed by the **adenovirus tripartite leader** sequence, a multiple cloning site for the insertion of the gene of interest and a polyadenylation sequence. To enable expression of peptides to be secreted into the tissue culture medium or to be incorporated into the cell membrane, several modifications have been introduced into such vectors: stop codons in all three reading frames were inserted at the end of the multiple cloning site and a DNA sequence coding for a signal peptide for transport through the endoplasmic reticulum (ER) was introduced downstream of the **adenovirus tripartite leader** sequence followed by two unique restriction enzyme recognition sites. A protocol is described that allows fast and easy cloning of

peptide-coding regions, i.e., PCR products, for expression and secretion. The transport of a genetically engineered chimeric transmembrane protein connected to this ER leader sequence was as efficient as that of the original protein from which the ER sequence has been derived. These universal vectors can also be used for the easy construction of any chimeric transmembrane or secretion proteins.

L6 ANSWER 9 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:558835 CAPLUS
DOCUMENT NUMBER: 123:162133
TITLE: Highly efficient eukaryotic gene expression vectors for peptide secretion
AUTHOR(S): Chu, Te-Hua Tearina; Martinez, Idali; Olson, Paul; Dornburg, Ralph
CORPORATE SOURCE: Univ. Med. Dent. New Jersey, Piscataway, NJ, USA
SOURCE: BioTechniques (1995), 18(5), 890-9
CODEN: BTNQDO; ISSN: 0736-6205
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Recently, we constructed a series of highly efficient universal eukaryotic gene expression vectors (Sheay et al., BioTechniques 15:856-862, 1993). Such vectors contain a viral promoter and enhancer followed by the **adenovirus tripartite leader** sequence, a multiple cloning site for the insertion of the gene of interest and a polyadenylation sequence. To enable expression of peptides to be secreted into the tissue culture medium or to be incorporated into the cell membrane, several modifications have been introduced into such vectors: stop codons in all three reading frames were inserted at the end of the multiple cloning site and a DNA sequence coding for a signal peptide for transport through the endoplasmic reticulum (ER) was introduced downstream of the **adenovirus tripartite leader** sequence followed by two unique restriction enzyme recognition sites. A protocol is described that allows fast and easy cloning of peptide-coding regions, i.e., PCR products, for expression and secretion. The transport of a genetically engineered chimeric transmembrane protein connected to this ER leader sequence was as efficient as that of the original protein from which the ER sequence has been derived. These universal vectors can also be used for the easy construction of any chimeric transmembrane or secretion proteins.

L6 ANSWER 10 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:46826 CAPLUS
DOCUMENT NUMBER: 120:46826
TITLE: Downstream insertion of the **adenovirus tripartite leader** sequence

enhances expression in universal eukaryotic vectors

AUTHOR(S): Sheay, W.; Nelson, S.; Martinez, I.; Chu, T. H. T.; Bhatia, S.; Dornburg, R.

CORPORATE SOURCE: Robert Wood Johnson Med. Sch., Univ. Med. Dent. New Jersey, Piscataway, NJ, USA

SOURCE: BioTechniques (1993), 15(5), 856, 858, 860-2
CODEN: BTNQDO; ISSN: 0736-6205

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A series of universal eukaryotic gene expression vectors was constructed. All vectors contain a viral promoter and enhancer, a polyliner for insertion of the gene of interest and a polyadenylation sequence. To enhance translation, the authors inserted the tripartite leader sequence of an adenovirus downstream of the promoter. Using the chloramphenicol acetyl transferase (cat) gene as a marker, the authors show that the strength of various promoters/enhancers in different cell lines differed by two orders of magnitude. The presence of the tripartite leader increased the efficiency of gene expression up to 18-fold. The level of increase is promoter specific and is most likely influenced by addnl. sequences flanking the tripartite leader sequence.

L6 ANSWER 11 OF 27 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1992:506518 CAPLUS

DOCUMENT NUMBER: 117:106518

TITLE: Dependence of the **adenovirus tripartite leader** on the p220 subunit of eukaryotic initiation factor 4F during in vitro translation. Effect of p220 cleavage by foot-and-mouth-disease-virus L-protease on in vitro translation

AUTHOR(S): Thomas, Adri A. M.; Scheper, Gert C.; Kleijn, Miranda; De Boer, Mariska; Voorma, Harry O.

CORPORATE SOURCE: Dep. Mol. Cell Biol., Univ. Utrecht, Utrecht, 3584 CH, Neth.

SOURCE: Eur. J. Biochem. (1992), 207(2), 471-7
CODEN: EJBCAI; ISSN: 0014-2956

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **adenovirus tripartite leader** (TPT) 5'- untranslated region (5'UTR) allows translation in poliovirus-infected cells, in which the p220 subunit of eukaryotic initiation factor 4F is degraded. This p220-independent translation was investigated by measuring in vitro translation in a reticulocyte lysate of a reporter gene, chloramphenicol acetyltransferase, coupled to the TPT 5'UTR. The p220 subunit was degraded by translation of a foot-and-mouth disease L-protease construct.

Surprisingly, the TPT 5'UTR was dependent on intact p220, as are other naturally capped mRNA species. Translation of encephalomyocarditis virus RNA was p220 independent, as expected from its ability to support internal, cap-independent initiation. In vitro protein synthesis expts. with purified initiation factors confirmed the dependence of TPT mRNA translation on eukaryotic initiation factor 4F. The relationship between adenovirus TPT 5'UTR-directed translation and poliovirus-induced host cell shut-off is discussed.

L6 ANSWER 12 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:136932 CAPLUS
DOCUMENT NUMBER: 114:136932
TITLE: Characterization of cap-binding protein complex-independent translation by the **adenovirus tripartite leader** 5' noncoding region: investigation of secondary structure and RNA interactions
AUTHOR(S): Dolph, Patrick Jay
CORPORATE SOURCE: New York Univ., New York, NY, USA
SOURCE: (1990) 142 pp. Avail.: Univ. Microfilms Int., Order No. DA9025110
From: Diss. Abstr. Int. B 1991, 51(7), 3250
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L6 ANSWER 13 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1990:453284 CAPLUS
DOCUMENT NUMBER: 113:53284
TITLE: Translation by the **adenovirus tripartite leader**: elements which determine independence from cap-binding protein complex
AUTHOR(S): Dolph, Patrick J.; Huang, Jiaoti; Schneider, Robert J.
CORPORATE SOURCE: Med. Cent., New York Univ., New York, NY, 10016, USA
SOURCE: J. Virol. (1990), 64(6), 2669-77
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **adenovirus tripartite leader** is a 200-nucleotide-long 5' noncoding region which facilitates translation of viral mRNAs at late times after infection. The tripartite leader also confers the ability to initiate translation independent of the requirement for cap-binding protein complex or

eIF-4F without any requirement for adenovirus gene products. To elucidate the manner by which the tripartite leader functions, the primary determinants of leader activity were investigated in vivo by testing a series of mutations expressed from transfected plasmids. The results of these expts. indicate that the tripartite leader does not promote internal ribosome binding, at least in a manner recently described for picornavirus mRNAs. In addn., despite an unusual arrangement of sequences complementary to the 3' end of 18S rRNA in the tripartite leader, no evidence was found for involvement in its translation activity. Instead, these results are consistent with a model in which much of the first leader is maintained in an unstructured conformation which dets. the ability of the tripartite leader to facilitate translation and bypass a normal requirement for eIF-4F activity. Several possible translation models are discussed, as well as the implications for translation of late viral mRNAs.

L6 ANSWER 14 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1990:211631 CAPLUS
DOCUMENT NUMBER: 112:211631
TITLE: High level expression of the envelope glycoproteins of the human immunodeficiency virus type 1 in the presence of rev gene using helper-independent adenovirus type 7 recombinants
AUTHOR(S): Chanda, Pranab K.; Natuk, Robert J.; Mason, Bruce B.; Bhat, Bheem M.; Greenberg, Lynda; Dheer, Surendra K.; Molnar-Kimber, Katherine L.; Mizutani, Satoshi; Lubeck, Michael D.; et al.
CORPORATE SOURCE: Biotechnol. Microbiol. Div., Wyeth-Ayerst Res., Philadelphia, PA, 19101, USA
SOURCE: Virology (1990), 175(2), 535-47
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effect of the rev (arts/trs) gene on the level of HIV-1 envelope (env) expression using recombinant adenovirus was investigated. Recombinant adenoviruses expressing either the envelope or the rev gene of the human immunodeficiency virus type 1 (HIV-1) were constructed by inserting the gene into an expression cassette. The expression cassette contained the adenovirus type 7 major late promoter, followed by leader 1 of the **adenovirus tripartite leader** and a portion of intron between leaders 1 and 2, leaders 2 and 3, and a hexon polyadenylation signal. The cassette was then inserted at the terminal region between the E4 and ITR regions of the adenovirus 7 genome with a concomitant E3 region deletion (80-87 m.u.). A549 cells infected with the recombinant virus contg. the env gene produced the envelope glycoproteins gp160, gp120, and gp41. HIV-1 envelope gene

expression was greatly enhanced (20-50-fold) in the cells that were simultaneously infected with the recombinant adenovirus contg. the rev gene, as measured by ELISA and Western blotting. This effect was obsd. despite the lack of the 5' down splice site for rev and seems to be post-transcriptional. Another recombinant adenovirus which contains both the rev and the env genes was constructed by inserting the rev gene in the deleted E3 region and the env gene in the terminal cassette. This double recombinant virus expressed high levels of env antigen in A549 cells similar to those attained upon co-infection with 2 sep. recombinant viruses contg. the rev or env gene. Furthermore, the rev gene nucleotide sequence could be altered without altering the amino acid sequence, and theamino acid sequence could be truncated by 17 amino acids from the C-terminus with no effect on rev function.

L6 ANSWER 15 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1989:452621 CAPLUS
DOCUMENT NUMBER: 111:52621
TITLE: Secondary structure analysis of **adenovirus tripartite leader**
AUTHOR(S): Zhang, Yan; Dolph, Patrick J.; Schneider, Robert J.
CORPORATE SOURCE: Kaplan Cancer Cent., New York Univ., New York, NY, 10016, USA
SOURCE: J. Biol. Chem. (1989), 264(18), 10679-84
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB RNA secondary structure anal. was performed to understand the translation function of the **adenovirus tripartite leader**, a 200-nucleotide 5' noncoding region on all late viral mRNAs. The tripartite leader facilitates the translation of viral mRNAs at late but not early times after infection and eliminates the normal requirement for the eukaryotic initiation factor 4F or cap binding protein complex. Secondary structures were detd. by probing 5' or 3' end-labeled tripartite leader RNAs under nondenaturing conditions with various single strand-specific nucleases, and the information was used to generate a potential model structure. The resulting structure is attractive since it may explain the unusual translation behavior conferred by the tripartite leader. The 1st leader segment is predominantly single-stranded, a property consistent with the ability to enhance translation and provide independence from cap binding protein complex. In contrast, the remaining 2 leader segments form a moderately stable base-paired structure, except for a large hairpin loop. To confirm these findings, the secondary structure of the tripartite leader was also probed when it was attached to a large segment of a mRNA and was

very similar to that of the individual leader RNA. These findings suggest several possible mechanisms to account for the translation activity of the tripartite leader.

L6 ANSWER 16 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1989:186935 CAPLUS
DOCUMENT NUMBER: 110:186935
TITLE: Initiation of protein synthesis by internal entry of ribosomes into the 5' nontranslated region of encephalomycarditis virus RNA in vivo
AUTHOR(S): Jang, Sung K.; Davies, Monique V.; Kaufman, Randal J.; Wimmer, Eckard
CORPORATE SOURCE: Sch. Med., State Univ. New York, Stony Brook, NY, 11794-8621, USA
SOURCE: J. Virol. (1989), 63(4), 1651-60
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Expression vectors that yield mono-, di-, and tricistronic mRNAs upon transfection of COS-1 cells were used to assess the influence of the 5' nontranslated regions (5' NTRs) on translation of reporter genes. A segment of the 5' NTR of encephalomyocarditis virus (EMCV) allowed translation of an adjacent downstream reporter gene (CAT) regardless of its position in th mRNAs. A deletion in the EMCV 5' NTR abolishes this effect. Poliovirus infection completely inhibits translation of the first cistron of a dicistronic mRNA that is preceded by the capped globin 5'NTR, whereas the second cistron preceded by the EMCV 5' NTR is still translated. It is concluded that the EMCV 5' NTR contains an internal ribosomal entry site that allows cap-independent initiation of translation. The mRNA contg. the **adenovirus tripartite leader** is also resistant to inhibition of translation by poliovirus.

L6 ANSWER 17 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:449300 CAPLUS
DOCUMENT NUMBER: 109:49300
TITLE: The **adenovirus tripartite leader** may eliminate the requirement for cap-binding protein complex during translation initiation
AUTHOR(S): Dolph, Patrick J.; Racaniello, Vincent; Villamarin, Alicia; Palladino, Francesca; Schneider, Robert J.
CORPORATE SOURCE: Dep. Biochem., New York Univ. Med. Cent., New York, NY, 10016, USA
SOURCE: J. Virol. (1988), 62(6), 2059-66
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **adenovirus tripartite leader** is a 200-nucleotide 5' noncoding region that has been identified on all late viral mRNAs. This segment is required for preferential translation of viral mRNAs at late times during infection. Tripartite leader-contg. mRNAs were shown to exhibit little if any requirement for intact cap-binding protein complex, a property previously established only for uncapped poliovirus mRNAs and capped mRNAs with minimal secondary structure. The tripartite leader also permits the translation of mRNAs in poliovirus infected cells in the apparent absence of active cap-binding protein complex and does not require any adenovirus gene products for this activity. The preferential translation of viral late mRNAs may involve this unusual property.

L6 ANSWER 18 OF 27 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1988:401552 CAPLUS

DOCUMENT NUMBER: 109:1552

TITLE: Efficient transcription, not translation, is dependent on **adenovirus tripartite leader** sequences at late times of infection

AUTHOR(S): Alonso-Caplen, Firelli V.; Katze, Michael G.; Krug, Robert M.

CORPORATE SOURCE: Grad. Program. Mol. Biol., Mem. Sloan-Kettering Cancer Cent., New York, NY, 10021, USA

SOURCE: J. Virol. (1988), 62(5), 1606-16
CODEN: JOVIAM; ISSN: 0022-538X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB To det. whether the tripartite leader is required for efficient translation in adenovirus-infected cells at late times of infection, recombinant adenoviruses contg. the influenza virus nucleocapsid protein (NP) gene expressed under the control of the adenovirus major late promoter (MLP) were constructed. The NP gene was chosen because previous results showed that the influenza virus NP mRNA was an extremely effective initiator of translation in cells which were superinfected with influenza virus at late times of adenovirus infection. The NP gene in the adenovirus recombinants was inserted downstream of an MLP that replaced part of the early (E1A) region. The resulting NP mRNAs either lacked any tripartite leader sequences or contained at their 5' ends various portions of the tripartite leader: 33, 172, or all 200 nucleotides of the leader. The relative amts. of the NP protein synthesized by the recombinants were directly proportional to the amts. of the NP mRNA made, indicating that the presence of 5' tripartite leader sequences did not enhance the translation of NP mRNA. In addn., the sizes of the polysomes contg. NP mRNA were not increased by the presence of tripartite

leader sequences, indicating that the initiation of translation was not enhanced by these sequences. On the other hand, the presence of tripartite leader sequences immediately downstream of the MLP did enhance the transcription of the inserted NP gene, as shown by Northern (RNA) anal. of in vivo NP mRNA levels and by in vitro runoff assays with isolated nuclei. These results indicate that more than 33 nucleotides of the first leader segment of the tripartite leader are required for optimal transcription from the MLP.

L6 ANSWER 19 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:181131 CAPLUS
DOCUMENT NUMBER: 108:181131
TITLE: The **adenovirus tripartite leader** sequence can alter nuclear and cytoplasmic metabolism of a non-adenovirus mRNA within infected cells
AUTHOR(S): Moore, Mary A.; Shenk, Thomas
CORPORATE SOURCE: Dep. Mol. Biol., Princeton Univ., Princeton, NJ, 08544, USA
SOURCE: Nucleic Acids Res. (1988), 16(5, Pt. B), 2247-62
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English
AB All mRNAs encoded by the adenovirus major late transcription unit share a common 5' noncoding region, 200 nucleotides in length, termed the tripartite leader sequence. To assess the function of the tripartite leader, recombinant viruses were prepd. which carried either a bona fide herpes simplex virus thymidine kinase gene or a modified thymidine kinase gene whose normal 5' noncoding domain was replaced with the adenovirus leader sequence. The tripartite leader simultaneously decreased the nuclear half-life and increased the cytoplasmic half-life of the thymidine kinase-specific mRNA. The tripartite leader stabilized the non-adenovirus mRNA only within the environment of an adenovirus-infected cell during the late phase of the infectious cycle.

L6 ANSWER 20 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1987:612886 CAPLUS
DOCUMENT NUMBER: 107:212886
TITLE: Post-transcriptional control of an induced cellular gene and a non-adenovirus gene bearing the **adenovirus tripartite leader** late after adenovirus infection
AUTHOR(S): Moore, Mary Ann
CORPORATE SOURCE: State Univ. New York, Stony Brook, NY, USA
SOURCE: (1986) 103 pp. Avail.: Univ. Microfilms Int., Order No. DA8710646

From: Diss. Abstr. Int. B 1987, 48(4), 967
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L6 ANSWER 21 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1986:566240 CAPLUS
DOCUMENT NUMBER: 105:166240
TITLE: Expression of hepatitis-B surface antigen with a recombinant adenovirus
AUTHOR(S): Davis, Alan R.; Kostek, Beverley; Mason, Bruce B.; Hsiao, Chu Lai; Morin, John; Barton, Joan; Dheer, Durendra K.; Zandle, Gordon; Hung, Paul P.
CORPORATE SOURCE: Microbiol. Div., Wyeth Lab., Inc., Philadelphia, PA, 19101, USA
SOURCE: Vaccines 86, New Approaches Immun., [Proc. Conf.] (1986), Meeting Date 1985, 283-7. Editor(s): Brown, Fred; Chanock, Robert M.; Lerner, Richard Alan. Cold Spring Harbor Lab.: Cold Spring Harbor, N. Y.
CODEN: 55ENAN
DOCUMENT TYPE: Conference
LANGUAGE: English
AB An infectious lipid adenovirus 2 recombinant vector was constructed which expresses the hepatitis virus surface antigen (HBsAg) under the control of the adenovirus major late promoter in infected human cells. The HBsAg polypeptide was glycosylated and was secreted from the adenovirus-infected 293 cells. Two recombinant viruses, HM1 and HM2 were also constructed. These viruses contained the tripartite leader segment in their mRNA. HM2 virus elicited a 70-fold increase in HBsAg prodn. over .DELTA.E1H9 virus, which does not contain the entire tripartite leader. Thus, high-level prodn. of HBsAg occurs late in adenovirus infection if the HBsAg-specific mRNA is made from a DNA where the **adenovirus tripartite leader** sequence precedes the HBsAg gene.

L6 ANSWER 22 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1985:433012 CAPLUS
DOCUMENT NUMBER: 103:33012
TITLE: Overproduction of the protein product of a nonselected foreign gene carried by an adenovirus vector
AUTHOR(S): Yamada, Masao; Lewis, John A.; Grodzicker, Terri
CORPORATE SOURCE: Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1985), 82(11), 3567-71

CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A recombinant adenovirus was constructed that carries the herpes simplex virus type I gene for thymidine kinase (EC 2.7.1.21) [9002-06-6] and expresses thymidine kinase under control of adenovirus major late promoter. A DNA fragment carrying thymidine kinase-coding sequences but lacking the thymidine kinase promoter was sandwiched between a piece of adenoviral DNA and SV40 virus early DNA on a plasmid. The aligned fragment was then inserted into the adenoviral genome, replacing internal adenoviral DNA. Hybrid viruses carrying the thymidine kinase gene were obtained by selecting for viruses that express SV40 40 tumor antigen (T antigen) in monkey cells. The thymidine kinase gene was positioned in the 3rd segment of the **adenovirus tripartite leader** downstream from the major late promoter by in vivo DNA recombination between the duplicated adenoviral sequences present in the plasmid insert and the viral vector. Levels of thymidine kinase activity in human or monkey cells infected with this hybrid virus were several times higher than in cells infected with herpes simplex virus. Infected cells produced thymidine kinase protein at very high levels, similar to those found for adenovirus late major capsid proteins. The thymidine kinase protein represented 10% of the newly synthesized protein in late infected cells and accumulated to represent 1% of total cell protein under optimal conditions. This vector system offers a procedure by which a variety of gene products that are biol. active and properly modified can be produced at high levels in mammalian cells.

L6 ANSWER 23 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1985:144098 CAPLUS
DOCUMENT NUMBER: 102:144098
TITLE: Effect of the tripartite leader on synthesis of a non-viral protein in an adenovirus 5 recombinant
AUTHOR(S): Berkner, Kathleen L.; Sharp, Phillip A.
CORPORATE SOURCE: Cent. Cancer Res., Massachusetts Inst. Technol., Cambridge, MA, 02139, USA
SOURCE: Nucleic Acids Res. (1985), 13(3), 841-57
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The EIa region of an adenovirus 5 recombinant was substituted by a modular gene encoding dihydrofolate reductase (DHFR) [9002-03-3]. In this recombinant, the mouse DHFR cDNA was positioned behind sequences of the major late promoter and the complete tripartite leader. The leader sequences end in the normal 5' splice site of the 3rd leader, so that RNA splicing joins the tripartite leader to

a 3' splice site immediately upstream of the DHFR cDNA. At late stages of infection, high levels of DHFR mRNAs were synthesized. At early times in the late stage, this mRNA was efficiently translated; however, at later times, translation of DHFR decreased probably due to poor competition with other late mRNAs. Synthesis of DHFR protein from an analogous adenovirus 5 recombinant contg. only the 1st late leader was studied in parallel. Equivalent levels of DHFR mRNA were expressed after infection with this recombinant virus; however, the efficiency of DHFR translation was .gtoreq.20 fold lower than that of the DHFR mRNA contg. the tripartite leader. This suggests that the tripartite leader sequence is important for translation in the late stage of infection. The adenovirus 5 recombinant contg. only the 1st leader vastly overexpresses polypeptide IX from a novel mRNA, formed by the splicing of the 1st leader in the modular DHFR gene to the 3' splice site in the EIb region. Cells infected with this recombinant synthesize very little normal mRNA from the EIb region. Coinfection of 293 cells with this recombinant and wild-type adenovirus 5 also results in decreased EIb mRNA synthesis. Perhaps the overprodn. of polypeptide IX suppresses mRNA expression from the EIb and IX promoter sites, probably by an autoregulation loop active during lytic growth.

L6 ANSWER 24 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1984:484771 CAPLUS
DOCUMENT NUMBER: 101:84771
TITLE: **Adenovirus tripartite leader** sequence enhances translation of mRNAs late after infection
AUTHOR(S): Logan, John; Shenk, Thomas
CORPORATE SOURCE: Health Sci. Cent., State Univ. New York, Stony Brook, NY, 11794, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1984), 81(12), 3655-9
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A series of adenovirus type 5 variants was constructed to probe the function of the tripartite leader sequence, a 200-nucleotide, 5'-noncoding sequence carried on the majority of late viral mRNAs. Recombinant plasmids were constructed that carried the major late transcriptional control region, which was followed by portions of the t ripartite leader sequence fused to the E1A coding region. These modified E1A genes were then rebuilt into intact viral chromosomes, and replaced the corresponding wild-type region. The leader segments had no effect on the translation of E1A mRNAs early after infection, but the tripartite leader significantly enhanced (5-fold) the efficiency with which the mRNAs were translated late after infection.

L6 ANSWER 25 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1983:28879 CAPLUS
DOCUMENT NUMBER: 98:28879
TITLE: Construction of adenovirus expression vectors by site-directed in vivo recombination
AUTHOR(S): Thummel, Carl; Tjian, Robert; Grodzicker, Terri
CORPORATE SOURCE: Dep. Biochem., Univ. California, Berkeley, CA, USA
SOURCE: J. Mol. Appl. Genet. (1982), 1(5), 435-46
CODEN: JMAGD2; ISSN: 0271-6801
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A method was developed for conveniently positioning foreign DNA at many preselected sites in the adenoviral genome by a combination of in vitro and in vivo recombination. Using this technique, a set of recombinant viruses that contain the SV40 A gene downstream from the **adenovirus tripartite leader** was constructed. One of these hybrid viruses (Ad-SVR26) contains the A gene close to and downstream from both the major late promoter and the 1st segment of the tripartite leader. The transcripts encoded by the inserted SV40 DNA are highly overproduced in infected cells; they initiate at the adenoviral late promoter and terminate at the SV40 polyadenylation site. Several novel splice acceptor sites in the SV40 sequences are used in the processing of the primary transcript to produce 6 different species of spliced RNA. The synthesis of T antigen in Ad-SVR26-infected cells requires the use of novel AUG initiation codons present within the SV40 coding region or adenoviral sequences that normally form part of the intron between the 1st and 2nd segments of the tripartite leader. The level of T antigen expression is not as high as the level of mRNA prodn. The usage of these new AUG triplets or the absence of the complete **adenovirus tripartite leader** sequence may account for the low efficiency of translation.

L6 ANSWER 26 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1982:467182 CAPLUS
DOCUMENT NUMBER: 97:67182
TITLE: Precise positioning of SV40 DNA in adenovirus expression vectors by a combination of in vitro and in vivo recombination
AUTHOR(S): Thummel, Carl S.; Tjian, Robert; Grodzicker, Terri
CORPORATE SOURCE: Dep. Biochem., Univ. California, Berkeley, CA, 94720, USA
SOURCE: Eukaryotic Viral Vectors, [Conf.] (1982), Meeting Date 1981, 181-6. Editor(s): Gluzman, Yakov. Cold Spring Harbor Lab.: Cold Spring

Harbor, N. Y.
CODEN: 48DVAI
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Construction of a hybrid virus, Ad-SVR26, that contains the SV40 A gene close to, but downstream from, the adenoviral major late promoter and the 1st segment of the **adenoviral tripartite leader**, is described. A technique that allowed the positioning of foreign DNA accurately within the adenoviral genome by site-directed homologous recombination was developed. Immunopptn. of exts. of cells contg. Ad-SVR26 with L18 or 3C5 monoclonal antibodies directed against the NH2-terminal and central regions, resp., of SV40 large T antigen yielded 2 proteins pptd. by 3C5 and 1 protein preferentially pptd. by L19. The smaller protein pptd. by 3C5 comigrated with wild-type T antigen; the other protein, which was also pptd. by L19, was .apprx.5000 daltons larger. Two adenovirus-SV40 hybrid mRNAs that encoded large T antigen were obsd; their lengths were 2150 and 2350 bases. Both were initiated from the adenoviral major late promoter and were present at .apprx.50-100-fold higher levels than found in SV40-infected cells. T antigen levels were not correspondingly high, probably due to inefficient translation. No small t antigen and no RNA encoding SV40 small t antigen was detected in cells contg. Ad-SVR26.

L6 ANSWER 27 OF 27 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1981:170901 CAPLUS
DOCUMENT NUMBER: 94:170901
TITLE: Expression of SV40 T antigen under control of adenovirus promoters
AUTHOR(S): Thummel, Carl; Tjian, Robert; Grodzicker, Terri
CORPORATE SOURCE: Dep. Biochem., Univ. California, Berkeley, CA, 94720, USA
SOURCE: Cell (Cambridge, Mass.) (1981), 23(3), 825-36
CODEN: CELLB5; ISSN: 0092-8674
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Adenovirus-SV40 recombinant viruses that express wild-type SV40 large T and small t antigens under the control of different adenovirus promoters were obtained. Hybrids were constructed in vitro with SV40 DNA that contains the entire early coding region but lacks the transcriptional promoter. Recombinants were isolated by a strong biol. selection for viruses that express SV40 T antigen. Anal. of several recombinant genomes indicates that they contain the SV40 A gene inserted in a variety of positions and orientations in the adenoviral genome. Moreover, the set of hybrid transcripts reveals an unexpected variety of splicing patterns. Some hybrid mRNAs transcribed from the adenovirus late promoter appear to

contain the **adenovirus tripartite leader** sequence. Other hybrid mRNAs were transcribed from adenovirus early promoters. All recombinant mRNAs contain intact SV40 early sequences that have normal splice patterns and produce wild-type T antigens. Biochem. characterization of SV40 T antigens overproduced by the hybrid viruses indicates that they are structurally indistinguishable from wild-type SV40 large T antigen and are functionally equiv. to the D2 protein.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 09:03:10 ON 07 MAY 2001)

L7 83 S L5
L8 79 S L7 NOT L3
L9 37 DUP REM L8 (42 DUPLICATES REMOVED)

L9 ANSWER 1 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 2000:580807 SCISEARCH
THE GENUINE ARTICLE: 337DP
TITLE: Leaky scanning is the predominant mechanism for translation of human papillomavirus type 16 E7 oncoprotein from E6/E7 bicistronic mRNA
AUTHOR: Stacey S N (Reprint); Jordan D; Williamson A J K; Brown M; Coote J H; Arrand J R
CORPORATE SOURCE: CHRISTIE HOSP NHS TRUST, PATERSON INST CANC RES, DEPT MOL BIOL, CANC RES CAMPAIGN, MANCHESTER M20 4BX, LANCS, ENGLAND (Reprint)
COUNTRY OF AUTHOR: ENGLAND
SOURCE: JOURNAL OF VIROLOGY, (AUG 2000) Vol. 74, No. 16, pp. 7284-7297.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
ISSN: 0022-538X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 75

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Human papillomaviruses (HPV) are unique in that they generate mRNAs that apparently can express multiple proteins from tandemly arranged open reading frames, The mechanisms by which this is achieved are uncertain and are at odds with the basic predictions of the scanning model for translation initiation. We investigated the unorthodox mechanism by which the E6 and E7 oncoproteins from human papillomavirus type 16 (HPV-16) can be translated from a single, bicistronic mRNA. The short E6 5' untranslated region (UTR) was shown to promote translation as efficiently as a UTR from Xenopus beta-globin. Insertion of a secondary structural element into the UTR inhibited both E6 and E7 expression, suggesting that E7

expression depends on ribosomal scanning from the 5' end of the mRNA, E7 translation was found to be cap dependent, but E6 was more dependent on capping and eIF4F activity than E7. Insertion of secondary structural elements at various points in the region upstream of E7 profoundly inhibited translation, indicating that scanning was probably continuous. Insertion of the E6 region between Renilla and firefly luciferase genes revealed little or no internal ribosomal entry site activity. However when E6 was located at the 5' end of the mRNA it permitted over 100-fold-higher levels of downstream cistron translation than did the Renilla open reading frame. Internal AUGs in the E6 region with strong or intermediate Kozak sequence contexts were unable to inhibit E7 translation, but initiation at the E7 AUG was efficient and accurate. These data support a model in which E7 translation is facilitated by an extreme degree of leaky scanning, requiring the negotiation of 13 upstream AUGs. Ribosomal initiation complexes which fail to initiate at the E6 start codon can scan through to the E7 AUG without initiating translation, but competence to initiate is achieved once the E7 AUG is reached. These findings suggest that the E6 region of HPV-16 comprises features that sponsor both translation of the E6 protein and enhancement of translation at a downstream site.

L9 ANSWER 2 OF 37 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
ACCESSION NUMBER: 2000:360545 BIOSIS
DOCUMENT NUMBER: PREV200000360545
TITLE: Gene immunization of mice with plasmid DNA expressing rabies virus glycoprotein.
AUTHOR(S): Fodor, I. (1); Kucsera, L.; Fodor, Nadja; Palfi, V.; Grabko, V. I.
CORPORATE SOURCE: (1) Center for Molecular Biology and Gene Therapy, Loma Linda University School of Medicine, 11085 Campus St., Loma Linda, CA, 92354 USA
SOURCE: Acta Veterinaria Hungarica, (2000) Vol. 48, No. 2, pp. 229-236. print.
ISSN: 0236-6290.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Gene immunization can be an effective vaccine strategy eliciting both humoral and cell-mediated immune responses. We constructed plasmid vectors expressing the full-length Vnukovo-32 rabies virus glycoprotein G under the control of CMV IE promoter and enhancer, **adenovirus tripartite leader** sequences
and poly A signal of SV40. The gene vaccines were evaluated for the ability to elicit neutralizing antibodies and to protect BALB/c mice against lethal rabies virus challenge. First, mice were injected intramuscularly (i.m.) into the left hind leg and by the intradermoplantar (i.d.p.) route with equal amounts of plasmid DNA

(0.25-0.1 mg). Two weeks later, immunization was boosted with an additional dose of the DNA. The immunized mice were challenged by intracerebral (i.c.) inoculation of CVS-27 (10-50 LD50) rabies virus. All mice produced anti-rabies virus neutralizing antibodies with a titre of gtoreq 1:45 after immunization with 0.1-0.4 mg of DNA. In challenge experiments, 83 to 91.6% protection was observed. These results confirm that a DNA vaccine could be a simple and effective solution for preventing the spread of rabies.

L9 ANSWER 3 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1999:733762 SCISEARCH
THE GENUINE ARTICLE: 238CK
TITLE: eIF4 initiation factors: Effectors of mRNA recruitment to ribosomes and regulators of translation
AUTHOR: Gingras A C (Reprint); Raught B; Sonenberg N
CORPORATE SOURCE: MCGILL UNIV, DEPT BIOCHEM, 3655 DRUMMOND ST, MONTREAL, PQ H3G 1Y6, CANADA (Reprint); MCGILL UNIV, CTR CANC, MONTREAL, PQ H3G 1Y6, CANADA
COUNTRY OF AUTHOR: CANADA
SOURCE: ANNUAL REVIEW OF BIOCHEMISTRY, (SEP 1999) Vol. 68, pp. 913-963.
Publisher: ANNUAL REVIEWS INC, 4139 EL CAMINO WAY, PO BOX 10139, PALO ALTO, CA 94303-0139.
ISSN: 0066-4154.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 383

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Eukaryotic translation initiation factor 4F (eIF4F) is a protein complex that mediates recruitment of ribosomes to mRNA. This event is the rate-limiting step for translation under most circumstances and a primary target for translational control. Functions of the constituent proteins of eIF4F include recognition of the mRNA 5' cap structure (eIF4E), delivery of an RNA helicase to the 5' region (eIF4A), bridging of the mRNA and the ribosome (eIF4G), and circularization of the mRNA via interaction with poly(A)-binding protein (eIF4G). eIF4 activity is regulated by transcription, phosphorylation, inhibitory proteins, and proteolytic cleavage. Extracellular stimuli evoke changes in phosphorylation that influence eIF4F activity, especially through the phosphoinositide 3-kinase (PI3K) and Ras signaling pathways. Viral infection and cellular stresses also affect eIF4F function. The recent determination of the structure of eIF4E at atomic resolution has provided insight about how translation is initiated and regulated. Evidence suggests that eIF4F is also implicated in malignancy and apoptosis.

L9 ANSWER 4 OF 37 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000106092 MEDLINE
DOCUMENT NUMBER: 20106092 PubMed ID: 10641338
TITLE: Induction of protective immunity in chickens immunised with plasmid DNA encoding infectious bursal disease virus antigens.
AUTHOR: Fodor I; Horvath E; Fodor N; Nagy E; Rencendorsh A; Vakharia V N; Dube S K
CORPORATE SOURCE: Agricultural Biotechnology Centre, Godollo, Hungary.. ifodor@som.llu.edu
SOURCE: ACTA VETERINARIA HUNGARICA, (1999) 47 (4) 481-92. Journal code: 27Q; 8406376. ISSN: 0236-6290.
PUB. COUNTRY: Hungary
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000218
Last Updated on STN: 20000218
Entered Medline: 20000210

AB Direct DNA inoculations were used to determine the efficacy of gene immunisation of chickens to elicit protective immune responses against infectious bursal disease virus (IBDV). The vp2 gene of IBDV strains GP40 and D78, and the vp2-vp4-vp3 encoding segment of strain D78 were cloned in an expression vector which consisted of human cytomegalovirus (HCMV) immediate early enhancer and promoter, **adenovirus tripartite leader** sequences and SV40 polyadenylation signal. For purification of vaccine-quality plasmid DNA from E. coli, an effective method was developed. Chickens were vaccinated by inoculation of DNA by two routes (intramuscular and intraperitoneal). Two weeks later, chickens were boosted with DNA, and at 2 weeks post-boost, they were challenged with virulent IBDV strain. Low to undetectable levels of IBDV-specific antibodies and no protection were observed with DNA encoding VP2. However, plasmids encoding VP2-VP4-VP3 induced IBDV-specific antibodies and protection in the chickens. DNA immunisation opens a new approach to the development of gene vaccines for chickens against infectious diseases.

L9 ANSWER 5 OF 37 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 97479812 MEDLINE
DOCUMENT NUMBER: 97479812 PubMed ID: 9339893
TITLE: Comparison of various expression plasmids for the induction of immune response by DNA immunization.
AUTHOR: Lee A H; Suh Y S; Sung J H; Yang S H; Sung Y C
CORPORATE SOURCE: Department of Life Science, School of Environmental Engineering, Pohang University of Science and

Technology, Korea.
SOURCE: MOLECULES AND CELLS, (1997 Aug 31) 7 (4) 495-501.
Journal code: CRQ; 9610936. ISSN: 1016-8478.
PUB. COUNTRY: KOREA
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 19971224
Entered Medline: 19971114

AB Intramuscular injection of plasmid DNA is an efficient method to introduce a foreign gene into a live animal. We investigated several factors affecting the gene transfer efficiency and the following immune response by intramuscular injection of plasmid DNA. When the strength of several highly efficient viral promoters was compared in muscle by using the chloramphenicol acetyltransferase (CAT) gene as an indicator, cytomegalovirus (CMV) immediate early promoter was found to be stronger than any other viral promoters including Rous sarcoma virus (RSV), murine leukemia virus (SL3-3) and simian virus 40 (SV40) early promoters. Inclusion of **adenovirus tripartite leader** (TPL) sequences and a synthetic intron in the 5' untranslated region of mRNA moderately stimulated the CAT expression. On the other hand, the expression of encephalomyocarditis virus (EMCV) VP1 gene was greatly enhanced by the TPL sequences and an intron. The level of humoral immune response by intramuscular injection of various VP1 expression plasmids was compared. The seroconversion rate was highly dependent on the strength of the expression vector. However, the ratio of IgG1 and IgG2a immune response was not significantly variable depending on the strength of the expression vector. Also, the efficiency of the sindbis virus-based DNA vector was examined for the gene expression and immune response. Although a high level of CAT expression was obtained in muscle by using this system, VP1 was not produced as much as the conventional expression vectors. Furthermore, little humoral immune response was elicited by intramuscular injection of VP1-expressing sindbis vector, suggesting that this system was not superior to the conventional vector for DNA immunization.

L9 ANSWER 6 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 96:701668 SCISEARCH
THE GENUINE ARTICLE: VH852
TITLE: CAP-BINDING PROTEIN (EUKARYOTIC INITIATION-FACTOR 4E) AND 4E-INACTIVATING PROTEIN BP-1 INDEPENDENTLY REGULATE CAP-DEPENDENT TRANSLATION
AUTHOR: FEIGENBLUM D; SCHNEIDER R J (Reprint)
CORPORATE SOURCE: NYU, SCH MED, DEPT BIOCHEM, NEW YORK, NY, 10016

(Reprint); NYU, SCH MED, DEPT BIOCHEM, NEW YORK, NY, 10016; NYU, SCH MED, KAPLAN CANC CTR, NEW YORK, NY, 10016
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (OCT 1996) Vol. 16, No. 10, pp. 5450-5457.
ISSN: 0270-7306.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 48

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Cap-dependent protein synthesis in animal cells is inhibited by heat shock, serum deprivation, metaphase arrest, and infection with certain viruses such as adenovirus (Ad). At a mechanistic level, translation of capped mRNAs is inhibited by dephosphorylation of eukaryotic initiation factor 4E (eLF-4E) (cap-binding protein) and its physical sequestration with the translation repressor protein BP-1 (PHAS-I. Dephosphorylation of BP-I blocks cap-dependent translation by promoting sequestration of eIF-4E. Here me show that heat shock inhibits translation of capped mRNAs bg simultaneously inducing dephosphorylation of eIF-4E and BP-1, suggesting that cells might coordinately regulate translation of capped mRNBs bg impairing both the activity and the availability of eIF-4E. Like heat shock, late Ad infection is shown to induce dephosphorylation of eIF-4E. However, in contrast to heat shock, Ad also induces phosphorylation of BP-1 and release of eIF-4E. BP-1 and eLF-4E can therefore act on cap-dependent translation in either a mutually antagonistic or cooperative manner, Three sets of experiments Further underscore this point: (i) rapamycin is shown to block phosphorylation of BP-1 without inhibiting dephosphorylation of eIF-4E induced by heat shock or Ad infection, (ii) eIF-4E is efficiently dephosphorylated during heat shock or Ad infection regardless of whether it is in a complex with BP-1, and (iii) BP-1 is associated with eIF-4E in vivo regardless of the state of eIF-4E phosphorylation. These and other studies establish that inhibition of cap-dependent translation does not obligatorily involve sequestration of eIF-4E by BP-1. Rather, translation is independently regulated by the phosphorylation states of eIF-4E and the 4E-binding protein, BP-1. In addition, these results demonstrate that BF-1 and eIF-4E can act either in concert or in opposition to independently regulate cap-dependent translation, We suggest that independent regulation of eIF-4E and BP-1 might finely regulate the efficiency of translation initiation or possibly control cap-dependent translation for fundamentally different purposes.

L9 ANSWER 7 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 96:546147 SCISEARCH

THE GENUINE ARTICLE: UX162
TITLE: INITIATION OF PROTEIN-SYNTHESIS IN EUKARYOTIC CELLS
AUTHOR: PAIN V M (Reprint)
CORPORATE SOURCE: UNIV SUSSEX, SCH BIOL SCI, BRIGHTON, E SUSSEX, ENGLAND (Reprint)
COUNTRY OF AUTHOR: ENGLAND
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (15 MAR 1996) Vol. 236, No. 3, pp. 747-771.
ISSN: 0014-2956.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 382

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB It is becoming increasingly apparent that translational control plays an important role in the regulation of gene expression in eukaryotic cells. Most of the known physiological effects on translation are exerted at the level of polypeptide chain initiation. Research on initiation of translation over the past five years has yielded much new information, which can be divided into three main areas: (a) structure and function of initiation factors (including identification by sequencing studies of consensus domains and motifs) and investigation of protein-protein and protein-RNA interactions during initiation; (b) physiological regulation of initiation factor activities and (c) identification of features in the 5' and 3' untranslated regions of messenger RNA molecules that regulate the selection of these mRNAs for translation. This review aims to assess recent progress in these three areas and to explore their interrelationships.

L9 ANSWER 8 OF 37 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 97080497 MEDLINE
DOCUMENT NUMBER: 97080497 PubMed ID: 8921842
TITLE: Nucleotide sequence of ovine **adenovirus tripartite leader** sequence and homologues of the IVa2, DNA polymerase and terminal proteins.
AUTHOR: Vrati S; Brookes D E; Boyle D B; Both G W
CORPORATE SOURCE: CSIRO Division of Biomolecular Engineering, North Ryde, N.S.W., Australia.
SOURCE: GENE, (1996 Oct 24) 177 (1-2) 35-41.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U31557; GENBANK-U40839
ENTRY MONTH: 199612

ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19990129
Entered Medline: 19961226

AB Ovine adenovirus OAV287 was previously isolated from sheep in Western Australia. Here we describe a portion of its genome between map units 10.3 and 31.7 which includes major ORFs for homologues of the IVa2 polypeptide and the DNA replication proteins, Terminal protein and DNA polymerase, as well as the N-terminal portion of the 52/55-kDa polypeptide. In addition, as a prelude to possible adaptation of this virus as a vector we have mapped the elements which make up the tripartite leader sequence of late mRNAs, thereby defining the probable location of the OAV major late promoter. In other human and animal adenovirus genomes, one or two VA RNA genes are encoded between the ORFs for Terminal protein and 52/55-kDa polypeptides. In OAV, these ORFs overlap, suggesting that if VA RNA genes are present, they may lie elsewhere in the OAV genome.

L9 ANSWER 9 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 95:342305 SCISEARCH
THE GENUINE ARTICLE: QX422
TITLE: HIGHLY EFFICIENT EUKARYOTIC GENE-EXPRESSION VECTORS FOR PEPTIDE SECRETION
AUTHOR: CHU T H T (Reprint); MARTINEZ I; OLSON P; DORNBURG R
CORPORATE SOURCE: UNIV MED & DENT NEW JERSEY, ROBERT WOOD JOHNSON MED SCH, DEPT MOLEC GENET & MICROBIOL, PISCATAWAY, NJ, 08854 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: BIOTECHNIQUES, (MAY 1995) Vol. 18, No. 5, pp. 892. ISSN: 0736-6205.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 20
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recently, we constructed a series of highly efficient universal eukaryotic gene expression vectors (Sheay et at, BioTechniques 15:856-862, 1993). Such vectors contain a viral promoter and enhancer followed by the **adenovirus tripartite leader** sequence, a multiple cloning site for the insertion of tire gene of interest and a polyadenylation sequence. To enable expression of peptides to be secreted into the tissue culture medium or to be incorporated into the cell membrane, several modifications have been introduced into such vectors: stop codons in all three reading frames were inserted at the end of the multiple cloning site and a DNA sequence coding So, a signal peptide for transport through the endoplasmatic reticulum (ER) was introduced downstream of the **adenovirus tripartite leader** sequence followed by two unique restriction enzyme recognition sites. A

protocol is described that allows fast and easy cloning of peptide-coding regions, i.e., PCR products, for expression and secretion. The transport of a genetically engineered chimeric transmembrane protein connected to this ER leader sequence was as efficient as that of the original protein from which the ER sequence has been derived. These universal vectors can also be used for the easy construction of any chimeric transmembrane or secretion proteins.

L9 ANSWER 10 OF 37 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 95344744 MEDLINE
DOCUMENT NUMBER: 95344744 PubMed ID: 7619496
TITLE: Highly efficient eukaryotic gene expression vectors for peptide secretion.
AUTHOR: Chu T H; Martinez I; Olson P; Dornburg R
CORPORATE SOURCE: University of Medicine, Dentistry of New Jersey, Piscataway, USA.
SOURCE: BIOTECHNIQUES, (1995 May) 18 (5) 890-6, 898-9.
Journal code: AN3; 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950911
Last Updated on STN: 19950911
Entered Medline: 19950831

AB Recently, we constructed a series of highly efficient universal eukaryotic gene expression vectors (Sheay et al., BioTechniques 15:856-862, 1993). Such vectors contain a viral promoter and enhancer followed by the **adenovirus tripartite leader** sequence, a multiple cloning site for the insertion of the gene of interest and a polyadenylation sequence. To enable expression of peptides to be secreted into the tissue culture medium or to be incorporated into the cell membrane, several modifications have been introduced into such vectors: stop codons in all three reading frames were inserted at the end of the multiple cloning site and a DNA sequence coding for a signal peptide for transport through the endoplasmatic reticulum (ER) was introduced downstream of the **adenovirus tripartite leader** sequence followed by two unique restriction enzyme recognition sites. A protocol is described that allows fast and easy cloning of peptide-coding regions, i.e., PCR products, for expression and secretion. The transport of a genetically engineered chimeric transmembrane protein connected to this ER leader sequence was as efficient as that of the original protein from which the ER sequence has been derived. These universal vectors can also be used for the easy construction of any chimeric transmembrane or secretion

proteins.

L9 ANSWER 11 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 95:660553 SCISEARCH
THE GENUINE ARTICLE: RV708
TITLE: M(7)GPPPG CAP DEPENDENCE FOR EFFICIENT TRANSLATION OF DROSOPHILA 70-KDA HEAT-SHOCK-PROTEIN (HSP70) MESSENGER-RNA
AUTHOR: SONG H J; GALLIE D R; DUNCAN R F (Reprint)
CORPORATE SOURCE: UNIV SO CALIF, SCH PHARM, DEPT MOLEC PHARMACOL & TOXICOL, 1985 ZONAL AVE, LOS ANGELES, CA, 90033 (Reprint); UNIV SO CALIF, SCH PHARM, DEPT MOLEC PHARMACOL & TOXICOL, LOS ANGELES, CA, 90033; UNIV CALIF RIVERSIDE, DEPT BIOCHEM, RIVERSIDE, CA, 92521; UNIV SO CALIF, SCH MED, DEPT MOLEC MICROBIOL & IMMUNOL, LOS ANGELES, CA, 00000
COUNTRY OF AUTHOR: USA
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (15 SEP 1995) Vol. 232, No. 3, pp. 778-788.
ISSN: 0014-2956.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 65
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB To investigate whether preferential translation of the heat-shock mRNAs occurs via cap-independent translation, the requirement for the m(7)GpppC cap structure for efficient translation of 70-kDa heat-shock-protein (Hsp70) mRNA was quantified by in vitro translation and by in vivo translation following electroporation. Hsp70 mRNA was transcribed in vitro with and without a cap structure. Translation in the rabbit reticulocyte or wheat germ lysate was reduced about 70% when the cap was absent. For comparison, translation of uncapped encephalomyocarditis virus 5'-untranslated-region-containing mRNA was equal to or greater than capped mRNA, whereas translation of several non-heat-shock mRNAs was reduced by 85-95% when capping was omitted. Cap-dependent translational stimulation of Hsp70 is not due to increased stability, is not a kinetic effect, and requires the methylated GpppG. To confirm the in vitro analyses, capped and uncapped mRNA were introduced into Drosophila tissue culture cells by electroporation, followed by heat shock. Paralleling the in vitro results, uncapped Hsp70 mRNA translation was 70-80% reduced relative to the capped form. Complementary experiments in which eIF-4 was inactivated in vitro using either m(7)GTP cap analogue or foot-and-mouth-disease virus L protease expression likewise indicated that the cap-dependent translation pathway is required for optimal Hsp mRNA translation. Since cellular Hsp70 mRNA translation

during heat shock is very efficient, it is unlikely that translation via a cap-independent pathway is the principal basis for preferential translation.

L9 ANSWER 12 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 96:107810 SCISEARCH
THE GENUINE ARTICLE: BE51H
TITLE: CAP-INDEPENDENT TRANSLATION AND INTERNAL INITIATION OF TRANSLATION IN EUKARYOTIC CELLULAR MESSENGER-RNA MOLECULES
AUTHOR: IIZUKA N (Reprint); CHEN C; YANG Q; JOHANNES G; SARNOW P
CORPORATE SOURCE: NAGOYA CITY UNIV, SCH MED, DEPT VIROL, NAGOYA, AICHI 467, JAPAN (Reprint); UNIV COLORADO, HLTH SCI CTR, DEPT BIOCHEM BIOPHYS & GENET, DENVER, CO, 80262; UNIV COLORADO, HLTH SCI CTR, DEPT MICROBIOL, DENVER, CO, 80262
COUNTRY OF AUTHOR: JAPAN; USA
SOURCE: CURRENT TOPICS IN MICROBIOLOGY AND IMMUNOLOGY, (1995) Vol. 203, pp. 155-177.
ISSN: 0070-217X.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: ENGLISH
REFERENCE COUNT: 116

L9 ANSWER 13 OF 37 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 95383855 MEDLINE
DOCUMENT NUMBER: 95383855 PubMed ID: 7655184
TITLE: Highly efficient eukaryotic gene expression vectors for peptide secretion.
AUTHOR: Chu T H; Martinez I; Olson P; Dornburg R
CORPORATE SOURCE: University of Medicine and Dentistry of New Jersey, Piscataway, USA.
SOURCE: PEPTIDE RESEARCH, (1995 Mar-Apr) 8 (2) 101-7.
Journal code: BE1; 8913494. ISSN: 1040-5704.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951013
Last Updated on STN: 19951013
Entered Medline: 19951002
AB Recently, we constructed a series of highly efficient universal eukaryotic gene expression vectors (Sheay et al., BioTechniques 15:856-862, 1993). Such vectors contain a viral promoter and enhancer followed by the **adenovirus tripartite leader** sequence, a multiple cloning site for the insertion

of the gene of interest and a polyadenylation sequence. To enable expression of peptides to be secreted into the tissue culture medium or to be incorporated into the cell membrane, several modifications have been introduced into such vectors: stop codons in all three reading frames were inserted at the end of the multiple cloning site and a DNA sequence coding for a signal peptide for transport through the endoplasmatic reticulum (ER) was introduced downstream of the **adenovirus tripartite leader** sequence
followed by two unique restriction enzyme recognition sites. A protocol is described that allows fast and easy cloning of peptide-coding regions, i.e., PCR products, for expression and secretion. The transport of a genetically engineered chimeric transmembrane protein connected to this ER leader sequence was as efficient as that of the original protein from which the ER sequence has been derived. These universal vectors can also be used for the easy construction of any chimeric transmembrane or secretion proteins.

L9 ANSWER 14 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 96:107803 SCISEARCH
THE GENUINE ARTICLE: BE51H
TITLE: CAP-DEPENDENT AND CAP-INDEPENDENT TRANSLATION - OPERATIONAL DISTINCTIONS AND MECHANISTIC INTERPRETATIONS
AUTHOR: JACKSON R J (Reprint); HUNT S L; REYNOLDS J E; KAMINSKI A
CORPORATE SOURCE: DEPT BIOCHEM, TENNIS COURT RD, CAMBRIDGE CB2 1QW, ENGLAND (Reprint)
COUNTRY OF AUTHOR: ENGLAND
SOURCE: CURRENT TOPICS IN MICROBIOLOGY AND IMMUNOLOGY, (1995) Vol. 203, pp. 1-29.
ISSN: 0070-217X.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: ENGLISH
REFERENCE COUNT: 111

L9 ANSWER 15 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 93:656118 SCISEARCH
THE GENUINE ARTICLE: MD348
TITLE: TRANSLATIONAL CONTROL BY INFLUENZA-VIRUS - SELECTIVE TRANSLATION IS MEDIATED BY SEQUENCES WITHIN THE VIRAL MESSENGER-RNA 5'-UNTRANSLATED REGION
AUTHOR: GARFINKEL M S; KATZE M G (Reprint)
CORPORATE SOURCE: UNIV WASHINGTON, SCH MED, DEPT MICROBIOL, SEATTLE, WA, 98195
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (25 OCT 1993) Vol. 268, No. 30, pp. 22223-22226.

ISSN: 0021-9258.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 31

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In cells infected by influenza virus type A, host cell protein synthesis declines rapidly and dramatically, while influenza viral protein synthesis occurs efficiently throughout infection. Previously, we had shown that the selective translation of influenza viral mRNAs in infected cells occurred in a cap-dependent manner and was due at least in part to structures inherent in the mRNAs. Using chimeras containing the noncoding and coding regions of cellular and viral mRNAs, we can now report that the selective translation is mediated by sequences within the 5'-untranslated regions (UTR) of the viral mRNAs. Polysome analysis confirmed that a 45-nucleotide sequence contained in the 5'-UTR of the influenza viral nucleocapsid protein was necessary and sufficient to allow the host cell translational machinery to discriminate between viral and cellular mRNAs. In reciprocal experiments in which the 5'-UTR of the cellular mRNA-secreted embryonic alkaline phosphatase replaced the nucleocapsid protein 5'-UTR, viral protein synthesis was inhibited in virus-infected cells, resembling host protein synthesis. Finally, we demonstrated that the 5'-UTR of another influenza viral mRNA, that encoding the nonstructural protein, also conferred resistance to the shutoff of protein synthesis in influenza virus-infected cells.

L9 ANSWER 16 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 93:444743 SCISEARCH
THE GENUINE ARTICLE: LM272
TITLE: TRANSLATION OF THE HEPATITIS-B VIRUS P-GENE BY RIBOSOMAL SCANNING AS AN ALTERNATIVE TO INTERNAL INITIATION
AUTHOR: FOUILLOT N; TLOUZEAU S; ROSSIGNOL J M (Reprint); JEANJEAN O
CORPORATE SOURCE: LAB BIOL MOLEC REPLICAT, CNRS, UPR 272, F-94800 VILLEJUIF, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: JOURNAL OF VIROLOGY, (AUG 1993) Vol. 67, No. 8, pp. 4886-4895.
ISSN: 0022-538X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 54

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The hepatitis B virus (HBV) P gene which encodes the reverse

transcriptase and other proteins required for replication is expressed on the bicistronic mRNA pregenome which also encodes the capsid protein in its first cistron. Recent results have suggested that the hepadnaviral P gene is translated by internal entry of ribosomes upstream from the P gene, in the overlapping C gene. Using a reporter gene fused to the HBV C or P gene, we demonstrate that the C sequence does not allow internal initiation of translation. Alternatively, our results support a model in which the HBV P gene is translated by ribosomes which scan from the capped extremity of the bicistronic mRNA pregenome. The mechanism by which the ribosomes scan past four AUGs before they initiate translation at the P AUG was analyzed. Our results show that these AUGs are skipped via two mechanisms: leaky scanning on AUGs in a weak or suboptimal initiation context and translation of an out-of-C-frame minicistron followed by reinitiation at P AUG. The minicistron translation allows ribosomes to bypass an AUG in a favorable context that would otherwise be used as a start codon for translation of a truncated capsid protein. Our results suggest that this elaborated scanning mechanism permits the coordinate expression of the HBV C and P genes on the viral bicistronic mRNA pregenome.

L9 ANSWER 17 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 93:323145 SCISEARCH
THE GENUINE ARTICLE: LB794
TITLE: MODIFICATION OF EUKARYOTIC INITIATION-FACTOR 4F DURING INFECTION BY INFLUENZA-VIRUS
AUTHOR: FEIGENBLUM D; SCHNEIDER R J (Reprint)
CORPORATE SOURCE: NYU MED CTR, DEPT BIOCHEM, NEW YORK, NY, 10016; NYU MED CTR, KAPLAN CANC CTR, NEW YORK, NY, 10016
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF VIROLOGY, (JUN 1993) Vol. 67, No. 6, pp. 3027-3035.
ISSN: 0022-538X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 73

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Influenza virus infection of cells is accompanied by a striking shutoff of cellular protein synthesis, resulting in the exclusive translation of viral mRNAs. The mechanism for control of cellular protein synthesis by influenza virus is poorly understood, but several translation properties of influenza virus mRNAs which are potentially involved have been described. Influenza virus mRNAs possess the surprising ability to translate in the presence of inhibitory levels of inactive (phosphorylated) eukaryotic initiation factor 2 (eIF-2). In addition, influenza virus mRNAs were shown to be capable of translating in cells during the late phase of

adenovirus infection but not in cells infected by poliovirus. Since both adenovirus and poliovirus facilitate virus-specific translation by impairing the activity of initiation factor eIF-4F (cap-binding protein complex) but through different mechanisms, we investigated the translation properties of influenza virus mRNAs in more detail. We show that influenza virus infection is associated with the significant dephosphorylation and inactivation of eIF-4E (cap-binding protein), a component of eIF-4F, and accordingly that influenza virus mRNAs possess a moderate ability to translate by using low levels of eIF-4F. We also confirm the ability of influenza virus mRNAs to translate in the presence of high levels of inactive (phosphorylated) eIF-2 but to a more limited extent than reported previously. We suggest a potential mechanism for the regulation of protein synthesis by influenza virus involving a decreased requirement for large pools of active eIF-4F and eIF-2.

L9 ANSWER 18 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 93:122975 SCISEARCH
THE GENUINE ARTICLE: KN579
TITLE: SELECTIVE DESTABILIZATION OF SHORT-LIVED MESSENGER-RNAS WITH THE GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR AU-RICH 3' NONCODING REGION IS MEDIATED BY A COTRANSLATIONAL MECHANISM
AUTHOR: AHARON T; SCHNEIDER R J (Reprint)
CORPORATE SOURCE: NYU MED CTR, DEPT BIOCHEM, NEW YORK, NY, 10016; NYU MED CTR, KAPLAN CANC CTR, NEW YORK, NY, 10016
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (MAR 1993) Vol. 13, No. 3, pp. 1971-1980.
ISSN: 0270-7306.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 63
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The 3' noncoding region element (AUUUA)n specifically targets many short-lived mRNAs for degradation. Although the mechanism by which this sequence functions is not yet understood, a potential link between facilitated mRNA turnover and translation has been implied by the stabilization of cellular mRNAs in the presence of protein synthesis inhibitors. We therefore directly investigated the role of translation on mRNA stability. We demonstrate that mRNAs which are poorly translated through the introduction of stable secondary structure in the 5' noncoding region are not efficiently targeted for selective destabilization by the (AUUUA). element. These results suggest that AUUUA-mediated degradation involves either a 5'-->3' exonuclease or is coupled to ongoing translation of the mRNA. To distinguish between these two possibilities, we

inserted the poliovirus internal ribosome entry site, which promotes internal ribosome initiation, downstream of the 5' secondary structure. Translation directed by internal ribosome binding was found to fully restore targeted destabilization of AUUUA-containing mRNAs despite the presence of 5' secondary structure. This study therefore demonstrates that selective degradation mediated by the (AUUUA)n element is coupled to ribosome binding or ongoing translation of the mRNA and does not involve 5'-to-3' exonuclease activity.

L9 ANSWER 19 OF 37 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 94092462 MEDLINE
DOCUMENT NUMBER: 94092462 PubMed ID: 8267981
TITLE: Downstream insertion of the **adenovirus tripartite leader** sequence enhances expression in universal eukaryotic vectors.
AUTHOR: Sheay W; Nelson S; Martinez I; Chu T H; Bhatia S; Dornburg R
CORPORATE SOURCE: Dept. of Microbiology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway 08854.
SOURCE: BIOTECHNIQUES, (1993 Nov) 15 (5) 856-62.
Journal code: AN3; 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
Report; (TECHNICAL REPORT)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940215
Last Updated on STN: 19980206
Entered Medline: 19940201

AB A series of universal eukaryotic gene expression vectors was constructed. All vectors contain a viral promoter and enhancer, a polylinker for insertion of the gene of interest and a polyadenylation sequence. To enhance translation, we inserted the tripartite leader sequence of an adenovirus downstream of the promoter. Using the chloramphenicol acetyl transferase (cat) gene as a marker, we show that the strength of various promoters/enhancers in different cell lines differed by two orders of magnitude. The presence of the tripartite leader increased the efficiency of gene expression up to 18-fold. The level of increase is promoter specific and is most likely influenced by additional sequences flanking the tripartite leader sequence.

L9 ANSWER 20 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 93:693608 SCISEARCH
THE GENUINE ARTICLE: MF887
TITLE: DOWNSTREAM INSERTION OF THE **ADENOVIRUS**

TRIPARTITE LEADER SEQUENCE
ENHANCES EXPRESSION IN UNIVERSAL EUKARYOTIC VECTORS

AUTHOR: SHEAY W; NELSON S; MARTINEZ I; CHU T H T; BHATIA S; DORNBURG R (Reprint)
CORPORATE SOURCE: UNIV MED & DENT NEW JERSEY, ROBERT WOOD JOHNSON MED SCH, DEPT MICROBIOL, 675 HOES LANE, PISCATAWAY, NJ, 08854
COUNTRY OF AUTHOR: USA
SOURCE: BIOTECHNIQUES, (NOV 1993) Vol. 15, No. 5, pp. 856. ISSN: 0736-6205.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 22
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A series of universal eukaryotic gene expression vectors was constructed All vectors contain a viral promoter and enhancer, a polylinker for insertion of the gene of interest and a polyadenylation sequence. To enhance translation, we inserted the tripartite leader sequence of an adenovirus downstream of the promoter Using the chloramphenicol acetyl transferase (cat) gene as a marker we show that the strength of various promoters/enhancers in different cell lines differed by two orders of magnitude. The presence of the tripartite leader increased the efficiency of gene expression up to 18-fold. The level of increase is promoter specific and is most likely influenced by additional sequences flanking the tripartite leader sequence.

L9 ANSWER 21 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 92:293837 SCISEARCH
THE GENUINE ARTICLE: HR854
TITLE: TRANSLATIONAL CONTROL BY INFLUENZA-VIRUS - SELECTIVE AND CAP-DEPENDENT TRANSLATION OF VIRAL MESSENGER-RNAS IN INFECTED-CELLS

AUTHOR: GARFINKEL M S; KATZE M G (Reprint)
CORPORATE SOURCE: UNIV WASHINGTON, SCH MED, DEPT MICROBIOL, SEATTLE, WA, 98195
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (05 MAY 1992) Vol. 267, No. 13, pp. 9383-9390. ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 56
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In cells infected by influenza virus type A, host protein synthesis undergoes a rapid and dramatic shutoff. To define the

molecular mechanisms underlying this selective translation, a transfection/infection protocol was developed utilizing viral and cellular cDNA clones. When COS-1 cells were transfected with cDNAs encoding nonviral genes and subsequently infected with influenza virus, protein expression from the exogenous genes was diminished, similar to the endogenous cellular genes. However, when cells were transfected with a truncated influenza viral nucleocapsid protein (NP-S) gene, the NP-S protein was made as efficiently in influenza virus infected cells as in uninfected cells, showing that the NP-S mRNA, although expressed independently of the influenza virus replication machinery, was still recognized as a viral and not a cellular mRNA. Northern blot analysis demonstrated that the selective blocks to nonviral protein synthesis were at the level of translation. Moreover, polysome experiments revealed that the translational blocks occurred at both the initiation and elongation stages of cellular protein synthesis. Finally, we utilized this transfection/infection system as well as double infection experiments to demonstrate that the translation of influenza viral mRNAs probably occurred in a cap-dependent manner as poliovirus infection inhibited influenza viral mRNA translation.

L9 ANSWER 22 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 92:319039 SCISEARCH
THE GENUINE ARTICLE: HU511
TITLE: THE EUKARYOTIC TRANSLATION INITIATION FACTOR-4E IS NOT MODIFIED DURING THE COURSE OF VACCINIA VIRUS-REPLICATION
AUTHOR: GIERMAN T M; FREDERICKSON R M; SONENBERG N; PICKUP D J (Reprint)
CORPORATE SOURCE: DUKE UNIV, MED CTR, DEPT MICROBIOL & IMMUNOL, DURHAM, NC, 27710; MCGILL UNIV, MCGILL CANC CTR, MONTREAL H3G 1Y6, QUEBEC, CANADA; MCGILL UNIV, DEPT BIOCHEM, MONTREAL H3G 1Y6, QUEBEC, CANADA
COUNTRY OF AUTHOR: USA; CANADA
SOURCE: VIROLOGY, (JUN 1992) Vol. 188, No. 2, pp. 934-937. ISSN: 0042-6822.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 46

L9 ANSWER 23 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 92:610852 SCISEARCH
THE GENUINE ARTICLE: JT170
TITLE: INTERACTION OF INITIATION-FACTORS WITH THE CAP STRUCTURE OF CHIMERIC MESSENGER-RNA CONTAINING THE 5'-UNTRANSLATED REGIONS OF SEMLIKI FOREST VIRUS-RNA IS RELATED TO TRANSLATIONAL EFFICIENCY

AUTHOR: BERBENBLOEMHEUVEL G; KASPERAITIS M A M; VANHEUGTEN H; THOMAS A A M; VANSTEEG H; VOORMA H O (Reprint)
CORPORATE SOURCE: UNIV UTRECHT, DEPT MOLEC & CELL BIOL, PADUALAAN 8, 3584 CH UTRECHT, NETHERLANDS
COUNTRY OF AUTHOR: NETHERLANDS
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (15 SEP 1992) Vol. 208, No. 3, pp. 581-587.
ISSN: 0014-2956.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 62

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Chimaeric chloramphenicol acetyltransferase (CAT) mRNA, containing the leader sequences of genomic 42S RNA and subgenomic 26S RNA of Semliki Forest virus (SFV) were synthesized by in-vitro transcription. These transcripts were translated with different efficiencies, as the authentic mRNA in SFV-infected cells. Therefore, they can be used as model mRNA species to study the mechanism underlying SFV-directed shut off of host protein synthesis. The interaction of translation initiation factors with the 5' cap structure was studied. Transcripts prepared in vitro using T7 RNA polymerase were capped and methylated posttranscriptionally with [P-32]-GTP and S-adenosyl-L-methionine to yield cap-labelled mRNA species. Irradiation with ultraviolet light of 26S CAT and 42S CAT transcripts, together with crude rabbit reticulocyte initiation factors, resulted in the cap-specific cross-linking of eukaryotic initiation factors (eIF) eIF-4E and eIF-4B. The relative binding efficiency of these two factors to the cap structure of the various transcripts was, however, markedly different; the cap structure present in 26S CAT mRNA interacted efficiently with cap-binding proteins, whereas the cap structure of 42S CAT mRNA hardly bound to these proteins. Comparable results were obtained under competitive conditions. Data are presented that the secondary structure close to the 5' cap structure determines the efficiency of recognition of the mRNA by these initiation factors. Using a chemical cross-linking assay, it was demonstrated that eIF-4F, and also eIF-4E, differentially interacted with the cap structure of the various transcripts. The data are discussed with respect to the possible mechanisms involved in SFV-induced shut off of host cell protein synthesis.

L9 ANSWER 24 OF 37 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 92339435 MEDLINE
DOCUMENT NUMBER: 92339435 PubMed ID: 1321714
TITLE: Dependence of the **adenovirus tripartite leader** on the p220 subunit of eukaryotic initiation factor 4F during in

vitro translation. Effect of p220 cleavage by foot-and-mouth-disease-virus L-protease on in vitro translation.

AUTHOR: Thomas A A; Scheper G C; Kleijn M; De Boer M; Voorma H O

CORPORATE SOURCE: Department of Molecular Cell Biology, University of Utrecht, The Netherlands.

SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1992 Jul 15) 207 (2) 471-7.
Journal code: EMZ; 0107600. ISSN: 0014-2956.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199208

ENTRY DATE: Entered STN: 19920911
Last Updated on STN: 20000303
Entered Medline: 19920825

AB The **adenovirus tripartite leader** (TPT) 5' untranslated region (5'UTR) allows translation in poliovirus-infected cells, in which the p220 subunit of eukaryotic initiation factor 4F is degraded. This p220-independent translation was investigated by measuring in vitro translation in a reticulocyte lysate of a reporter gene, chloramphenicol acetyltransferase, coupled to the TPT 5'UTR. The p220 subunit was degraded by translation of a foot-and-mouth-disease L-protease construct. Surprisingly, the TPT 5'UTR was dependent on intact p220, as are other naturally capped mRNA species. Translation of encephalomyocarditis virus RNA was p220 independent, as expected from its ability to support internal, cap-independent initiation. In vitro protein-synthesis experiments with purified initiation factors confirmed the dependence of TPT mRNA translation on eukaryotic initiation factor 4F. The relationship between adenovirus TPT-5'UTR-directed translation and poliovirus-induced host cell shut-off is discussed.

L9 ANSWER 25 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 91:438071 SCISEARCH

THE GENUINE ARTICLE: FZ332

TITLE: EFFECTS OF LONG 5' LEADER SEQUENCES ON INITIATION BY EUKARYOTIC RIBOSOMES INVITRO

AUTHOR: KOZAK M (Reprint)

CORPORATE SOURCE: UNIV MED & DENT NEW JERSEY, DEPT BIOCHEM, PISCATAWAY, NJ, 08854

COUNTRY OF AUTHOR: USA

SOURCE: GENE EXPRESSION, (1991) Vol. 1, No. 2, pp. 117-125.

DOCUMENT TYPE: Article; Journal

LANGUAGE: ENGLISH

REFERENCE COUNT: 40 Keyed
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Lengthening the 5' noncoding sequence on SP6-derived transcripts can increase their translational efficiency by an order of magnitude under some conditions of translation in reticulocyte lysates. This effect was observed upon reiterating three different synthetic oligonucleotides, the sequences of which were designed simply to preclude secondary structure. It seems unlikely that such arbitrarily designed sequences are recognized by sequence-specific translational enhancer proteins. Rather, long 5' leader sequences appear to accumulate extra 40S ribosomal subunits, which may account for their translational advantage. The buildup of 40S subunits on long, unstructured leader sequences is predicted by the scanning model for initiation. Leader sequences such as these may be ideal for in vitro expression vectors.

L9 ANSWER 26 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 90233103 EMBASE
DOCUMENT NUMBER: 1990233103
TITLE: Erratum: Translation by the **adenovirus tripartite leader**: Elements which determine independence from Cap-binding protein complex (Journal of Virology, Volume 64, no. 6, page 2674).
SOURCE: Journal of Virology, (1990) 64/8 (4042).
ISSN: 0022-538X CODEN: JOVIAM
COUNTRY: United States
DOCUMENT TYPE: Journal; Errata
FILE SEGMENT: 047 Virology
LANGUAGE: English

L9 ANSWER 27 OF 37 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 90244378 MEDLINE
DOCUMENT NUMBER: 90244378 PubMed ID: 1692346
TITLE: Translation by the **adenovirus tripartite leader**: elements which determine independence from cap-binding protein complex.
COMMENT: Erratum in: J Virol 1990 Aug;64(8):4042
AUTHOR: Dolph P J; Huang J T; Schneider R J
CORPORATE SOURCE: Kaplan Cancer Center, New York University Medical Center, New York 10016.
CONTRACT NUMBER: CA-42357 (NCI)
SOURCE: JOURNAL OF VIROLOGY, (1990 Jun) 64 (6) 2669-77.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199006
ENTRY DATE: Entered STN: 19900706
Last Updated on STN: 19960129
Entered Medline: 19900614

AB The **adenovirus tripartite leader** is a 200-nucleotide-long 5' noncoding region which facilitates translation of viral mRNAs at late times after infection. The tripartite leader also confers the ability to initiate translation independent of the requirement for cap-binding protein complex or eIF-4F without any requirement for adenovirus gene products. To elucidate the manner by which the tripartite leader functions, the primary determinants of leader activity were investigated in vivo by testing a series of mutations expressed from transfected plasmids. The results of these experiments indicate that the tripartite leader does not promote internal ribosome binding, at least in a manner recently described for picornavirus mRNAs. In addition, despite an unusual arrangement of sequences complementary to the 3' end of 18S rRNA in the tripartite leader, we could find no evidence for involvement in its translation activity. Instead, our results are consistent with a model in which much of the first leader is maintained in an unstructured conformation which determines the ability of the tripartite leader to facilitate translation and bypass a normal requirement for eIF-4F activity. Several possible translation models are discussed, as well as the implications for translation of late viral mRNAs.

L9 ANSWER 28 OF 37 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 90223999 MEDLINE
DOCUMENT NUMBER: 90223999 PubMed ID: 2183470
TITLE: High level expression of the envelope glycoproteins of the human immunodeficiency virus type I in presence of rev gene using helper-independent adenovirus type 7 recombinants.
AUTHOR: Chanda P K; Natuk R J; Mason B B; Bhat B M; Greenberg L; Dheer S K; Molnar-Kimber K L; Mizutani S; Lubeck M D; Davis A R; +
CORPORATE SOURCE: Biotechnology and Microbiology Division, Wyeth-Ayerst Research, Philadelphia, Pennsylvania 19101.
SOURCE: VIROLOGY, (1990 Apr) 175 (2) 535-47.
Journal code: XEA; 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199005
ENTRY DATE: Entered STN: 19900622
Last Updated on STN: 19970203

Entered Medline: 19900523

AB The effect of rev (art/trs) gene on the level of HIV-1 envelope (env) expression using recombinant adenovirus was investigated. Recombinant adenoviruses expressing either the envelope or the rev gene of the human immunodeficiency virus type 1 (HIV-1) were constructed by inserting the gene into an expression cassette. The expression cassette contained the adenovirus type 7 major late promoter, followed by leader 1 of the **adenovirus** **tripartite leader** and a portion of intron between leaders 1 and 2, leaders 2 and 3, and a hexon polyadenylation signal. The cassette was then inserted at the terminal region between the E4 and ITR regions of the adenovirus 7 genome with a concomitant E3 region deletion (80-87 m.u.). A549 cells infected with the recombinant virus containing the env gene produced the envelope glycoproteins gp160, gp120, and gp41. HIV-1 envelope gene expression was greatly enhanced (20- to 50-fold) in the cells that were simultaneously infected with the recombinant adenovirus containing the rev gene as measured by ELISA and Western blotting. Interestingly, this effect was observed despite the lack of the 5' down splice site for rev and seems to be post-transcriptional. Another recombinant adenovirus which contains both the rev and the env genes was constructed by inserting the rev gene in the deleted E3 region and the env gene in the terminal cassette. This double recombinant virus expressed high levels of env antigen in A549 cells similar to those attained upon co-infection with two separate recombinant viruses containing the rev or env gene. Furthermore, the rev gene nucleotide sequence could be altered without altering the amino acid sequence and its sequences truncated by 17 amino acids from the C-terminus had no effect of rev function.

DUPLICATE 11

L9 ANSWER 29 OF 37 MEDLINE
ACCESSION NUMBER: 89278139 MEDLINE
DOCUMENT NUMBER: 89278139 PubMed ID: 2732243
TITLE: Secondary structure analysis of **adenovirus** **tripartite leader**.
AUTHOR: Zhang Y; Dolph P J; Schneider R J
CORPORATE SOURCE: Department of Biochemistry, New York University Medical Center, New York 10016.
CONTRACT NUMBER: CA-42357 (NCI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jun 25) 264 (18) 10679-84.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198907
ENTRY DATE: Entered STN: 19900309

Searcher : Shears 308-4994

Last Updated on STN: 19990129
Entered Medline: 19890718

AB RNA secondary structure analysis was performed to understand the translation function of the **adenovirus tripartite leader**, a 200-nucleotide 5' noncoding region found on all late viral mRNAs. The tripartite leader facilitates the translation of viral mRNAs at late but not early times after infection and eliminates the normal requirement for the eukaryotic initiation factor 4F or cap binding protein complex. Secondary structures were determined by probing 5' or 3' end-labeled tripartite leader RNAs under nondenaturing conditions with various single strand-specific nucleases, and the information was used to generate a potential model structure. The resulting structure is attractive since it may explain the unusual translation behavior conferred by the tripartite leader. We demonstrate that the first leader segment is predominantly single-stranded, a property consistent with the ability to enhance translation and provide independence from cap binding protein complex. In contrast, the remaining two leader segments form a moderately stable base-paired structure, except for a large hairpin loop. To confirm these findings, the secondary structure of the tripartite leader was also probed when it was attached to a large segment of a messenger RNA and was found to be very similar to that of the individual leader RNA. These findings suggest several possible mechanisms to account for the translation activity of the tripartite leader.

L9 ANSWER 30 OF 37 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 89178851 MEDLINE
DOCUMENT NUMBER: 89178851 PubMed ID: 2538648
TITLE: Initiation of protein synthesis by internal entry of ribosomes into the 5' nontranslated region of encephalomyocarditis virus RNA in vivo.
AUTHOR: Jang S K; Davies M V; Kaufman R J; Wimmer E
CORPORATE SOURCE: Department of Microbiology, School of Medicine, State University of New York, Stony Brook 11794-8621.
CONTRACT NUMBER: AI-15122 (NIAID)
CA-28146 (NCI)
SOURCE: JOURNAL OF VIROLOGY, (1989 Apr) 63 (4) 1651-60.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198904
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 19980206
Entered Medline: 19890425

AB Expression vectors that yield mono-, di-, and tricistronic mRNAs

upon transfection of COS-1 cells were used to assess the influence of the 5' nontranslated regions (5'NTRs) on translation of reporter genes. A segment of the 5'NTR of encephalomyocarditis virus (EMCV) allowed translation of an adjacent downstream reporter gene (CAT) regardless of its position in the mRNAs. A deletion in the EMCV 5'NTR abolishes this effect. Poliovirus infection completely inhibits translation of the first cistron of a dicistronic mRNA that is preceded by the capped globin 5'NTR, whereas the second cistron preceded by the EMCV 5'NTR is still translated. We conclude that the EMCV 5'NTR contains an internal ribosomal entry site that allows cap-independent initiation of translation. mRNA containing the **adenovirus tripartite leader** is also resistant to inhibition of translation by poliovirus.

L9 ANSWER 31 OF 37 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 88189816 MEDLINE
DOCUMENT NUMBER: 88189816 PubMed ID: 3357776
TITLE: The **adenovirus tripartite leader** sequence can alter nuclear and cytoplasmic metabolism of a non-adenovirus mRNA within infected cells.
AUTHOR: Moore M A; Shenk T
CORPORATE SOURCE: Department of Molecular Biology, Princeton University, NJ 08544.
CONTRACT NUMBER: CA 41086 (NCI)
SOURCE: NUCLEIC ACIDS RESEARCH, (1988 Mar 25) 16 (5) 2247-62. Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198805
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880518

AB All mRNAs encoded by the adenovirus major late transcription unit share a common 5' noncoding region, 200 nucleotides in length, termed the tripartite leader sequence. To assess function of the tripartite leader, recombinant viruses were prepared which carried either a bona fide herpes simplex virus thymidine kinase gene or a modified thymidine kinase gene whose normal 5' noncoding domain was replaced with the adenovirus leader sequence. The tripartite leader simultaneously decreased the nuclear half-life and increased the cytoplasmic half-life of the thymidine kinase-specific mRNA. The tripartite leader stabilized the non-adenovirus mRNA only within the environment of an adenovirus-infected cell during the late phase of the infectious cycle.

L9 ANSWER 32 OF 37 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 88215035 MEDLINE
DOCUMENT NUMBER: 88215035 PubMed ID: 2835510
TITLE: The **adenovirus tripartite leader** may eliminate the requirement for cap-binding protein complex during translation initiation.
AUTHOR: Dolph P J; Racaniello V; Villamarin A; Palladino F; Schneider R J
CORPORATE SOURCE: Department of Biochemistry, New York University Medical Center, New York 10016.
CONTRACT NUMBER: AI 20017 (NIAID)
CA-42357 (NCI)
SOURCE: JOURNAL OF VIROLOGY, (1988 Jun) 62 (6) 2059-66.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198806
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880620

AB The **adenovirus tripartite leader** is a 200-nucleotide 5' noncoding region that is found on all late viral mRNAs. This segment is required for preferential translation of viral mRNAs at late times during infection. Most tripartite leader-containing mRNAs appear to exhibit little if any requirement for intact cap-binding protein complex, a property previously established only for uncapped poliovirus mRNAs and capped mRNAs with minimal secondary structure. The tripartite leader also permits the translation of mRNAs in poliovirus-infected cells in the apparent absence of active cap-binding protein complex and does not require any adenovirus gene products for this activity. The preferential translation of viral late mRNAs may involve this unusual property.

L9 ANSWER 33 OF 37 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 88188243 MEDLINE
DOCUMENT NUMBER: 88188243 PubMed ID: 2833610
TITLE: Efficient transcription, not translation, is dependent on **adenovirus tripartite leader** sequences at late times of infection.
AUTHOR: Alonso-Caplen F V; Katze M G; Krug R M
CORPORATE SOURCE: Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021.
CONTRACT NUMBER: AI-11772 (NIAID)
AI-22646 (NIAID)

09/482682

Alonso-Caplen et al

CA-08747 (NCI)

SOURCE: JOURNAL OF VIROLOGY, (1988 May) 62 (5) 1606-16.
Journal code: KCV; 0113724. ISSN: 0022-538X.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198805

ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880526

AB To determine whether the tripartite leader is required for efficient translation in adenovirus-infected cells at late times of infection, we constructed recombinant adenoviruses containing the influenza virus nucleocapsid protein (NP) gene expressed under the control of the adenovirus major late promoter (MLP). We chose the NP gene because previous results showed that the influenza virus NP mRNA was an extremely effective initiator of translation in cells which were superinfected with influenza virus at late times of adenovirus infection (M. G. Katze, B. M. Detjen, B. Safer, and R. M. Krug, Mol. Cell. Biol. 6:1741-1750, 1986). The NP gene in the adenovirus recombinants was inserted downstream of an MLP that replaced part of the early (E1A) region. The resulting NP mRNAs either lacked any tripartite leader sequences or contained at their 5' ends various portions of the tripartite leader: 33, 172, or all 200 nucleotides of the leader. The relative amounts of the NP protein synthesized by the recombinants were directly proportional to the amounts of the NP mRNA made, indicating that the presence of 5' tripartite leader sequences did not enhance the translation of NP mRNA. In addition, the sizes of the polysomes containing NP mRNA were not increased by the presence of tripartite leader sequences, indicating that the initiation of translation was not enhanced by these sequences. On the other hand, the presence of tripartite leader sequences immediately downstream of the MLP did enhance the transcription of the inserted NP gene, as shown by Northern (RNA) analysis of in vivo NP mRNA levels and by in vitro runoff assays with isolated nuclei. Our results indicate that more than 33 nucleotides of the first leader segment of the tripartite leader are required for optimal transcription from the MLP.

L9 ANSWER 34 OF 37 MEDLINE DUPLICATE 16

ACCESSION NUMBER: 85216542 MEDLINE

DOCUMENT NUMBER: 85216542 PubMed ID: 2987934

TITLE: Overproduction of the protein product of a nonselected foreign gene carried by an adenovirus vector.

AUTHOR: Yamada M; Lewis J A; Grodzicker T

CONTRACT NUMBER: CA13106 (NCI)

Searcher : Shears 308-4994

09/482682

Yamada et al

SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1985 Jun) 82 (11) 3567-71.
Journal code: PV3; 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

LANGUAGE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: English
ENTRY MONTH: Priority Journals
ENTRY DATE: 198507
Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19850722

AB We have constructed a recombinant adenovirus that carries the herpes simplex virus type I gene for thymidine kinase (EC 2.7.1.21) and expresses thymidine kinase under control of adenovirus major late promoter. A DNA fragment carrying thymidine kinase coding sequences but lacking the thymidine kinase promoter was sandwiched between a piece of adenoviral DNA and simian virus 40 early DNA on a plasmid. The aligned fragment was then inserted into the adenoviral genome, replacing internal adenoviral DNA. Hybrid viruses carrying the thymidine kinase gene were obtained by selecting for viruses that express simian virus 40 tumor antigen (T antigen) in monkey cells. The thymidine kinase gene was positioned in the third segment of the **adenovirus tripartite leader** downstream from the major late promoter by in vivo DNA recombination between the duplicated adenoviral sequences present in the plasmid insert and the viral vector. Levels of thymidine kinase activity in human or monkey cells infected with this hybrid virus were several times higher than in cells infected with herpes simplex virus. Infected cells produced thymidine kinase protein at very high levels, similar to those found for adenovirus late major capsid proteins. The thymidine kinase protein represented 10% of the newly synthesized protein in late infected cells and accumulated to represent 1% of total cell protein under optimal conditions. This vector system offers a procedure by which a variety of gene products that are biologically active and properly modified can be produced at high levels in mammalian cells.

DUPLICATE 17

L9 ANSWER 35 OF 37 MEDLINE
ACCESSION NUMBER: 84222018 MEDLINE
DOCUMENT NUMBER: 84222018 PubMed ID: 6587381
TITLE: **Adenovirus tripartite leader** sequence enhances translation of mRNAs late after infection.

AUTHOR: Logan J; Shenk T
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1984 Jun) 81 (12) 3655-9.

Searcher : Shears 308-4994

Journal code: PV3; 7505876. ISSN: 0027-8424.
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ENTRY MONTH: 198407
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AB A series of adenovirus type 5 variants was constructed to probe the function of the tripartite leader sequence, a 200-nucleotide, 5' noncoding sequence carried on the majority of late viral mRNAs. Recombinant plasmids were constructed that carried the major late transcriptional control region followed by portions of the tripartite leader sequence fused to the E1A coding region. These modified E1A genes were then rebuilt into intact viral chromosomes, replacing the corresponding wild-type region. The leader segments had no effect on the translation of E1A mRNAs early after infection, but the tripartite leader significantly enhanced (5-fold) the efficiency with which the mRNAs were translated late after infection.

L9 ANSWER 36 OF 37 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 83110642 MEDLINE
DOCUMENT NUMBER: 83110642 PubMed ID: 6296252
TITLE: Construction of adenovirus expression vectors by site-directed in vivo recombination.
AUTHOR: Thummel C; Tjian R; Grodzicker T
SOURCE: JOURNAL OF MOLECULAR AND APPLIED GENETICS, (1982) 1 (5) 435-46.
Journal code: IZT; 8109497. ISSN: 0271-6801.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198303
ENTRY DATE: Entered STN: 19900318
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AB We developed a method for conveniently positioning foreign DNA at many preselected sites in the adenoviral genome by a combination of in vitro and in vivo recombination. Using this technique, we constructed a set of recombinant viruses that contain the SV40 A gene downstream from the **adenovirus tripartite leader**. One of these hybrid viruses, Ad-SVR26, contains the A gene close to and downstream from both the major late promoter and the first segment of the tripartite leader. The transcripts encoded by the inserted SV40 DNA are highly overproduced in infected cells;

they initiate at the adenoviral late promoter and terminate at the SV40 polyadenylation site. Several novel splice acceptor sites in the SV40 sequences are used in the processing of the primary transcript to produce six different species of spliced RNA. The synthesis of T antigen in Ad-SVR26-infected cells requires the use of novel AUG initiation codons present within the SV40 coding region or adenoviral sequences that normally form part of the intron between the first and second segments of the tripartite leader. The level of T antigen expression is not as high as the level of mRNA production. The usage of these new AUG triplets or the absence of the complete **adenovirus tripartite leader** sequence may account for the low efficiency of translation.

L9 ANSWER 37 OF 37 MEDLINE DUPLICATE 19
ACCESSION NUMBER: 81186284 MEDLINE
DOCUMENT NUMBER: 81186284 PubMed ID: 7226231
TITLE: Expression of SV40 T antigen under control of adenovirus promoters.
AUTHOR: Thummel C; Tjian R; Grodzicker T
SOURCE: CELL, (1981 Mar) 23 (3) 825-36.
Journal code: CQ4; 0413066. ISSN: 0092-8674.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198107
ENTRY DATE: Entered STN: 19900316
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AB We have obtained novel adenovirus-SV40 recombinant viruses that express wild-type SV40 large T and small t antigens under the control of different adenovirus promoters. Hybrids were constructed in vitro with SV40 DNA that contains the entire early coding region but lacks the transcriptional promoter. Recombinants were isolated by a strong biological selection for viruses that express SV40 T antigen. Analysis of several recombinant genomes indicates that they contain the SV40 A gene inserted in a variety of positions and orientations in the adenoviral genome. Moreover, the set of hybrid transcripts reveals an unexpected variety of splicing patterns. Some hybrid mRNAs transcribed from the adenovirus late promoter appear to contain the **adenovirus tripartite leader** sequence. Other hybrid mRNAs were transcribed from adenovirus early promoters. All recombinant mRNAs contain intact SV40 early sequences that have normal splice patterns and produce wild-type T antigens. Biochemical characterization of SV40 T antigens overproduced by the hybrid viruses indicates that they are structurally indistinguishable from wild-type SV40 large T antigen and are

functionally equivalent to the D2 protein.

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